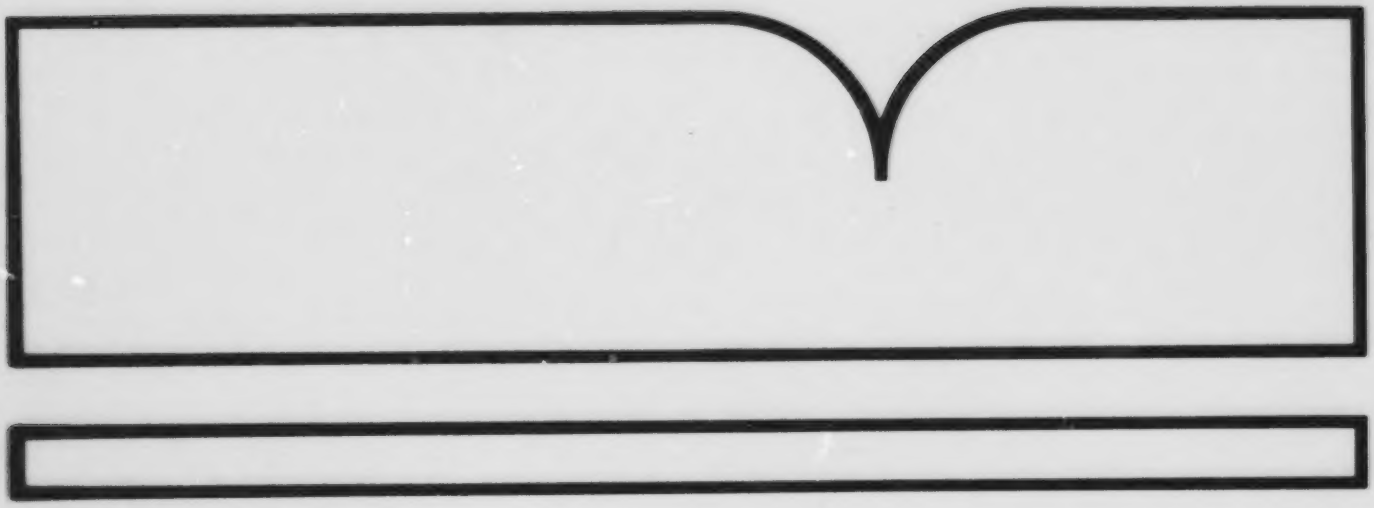
PB86-128576

Macrophysiological Assessment of Organometal Neurotoxicity

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Oct 85



U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-128576

EPA/600/D-85/271
October 1985

MACROPHYSIOLOGICAL ASSESSMENT OF ORGANOMETAL NEUROTOXICITY

by

Robert S. Dyer
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/D-85/271	2.	3. RECIPIENT'S ACCESSION NO. PB86 128576/AS
4. TITLE AND SUBTITLE Macrophysiological Assessment of Organometal Neurotoxicity		5. REPORT DATE October 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Robert S. Dyer		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS USEPA/HERL/NTD (MD-74B) RTP, NC 27711		10. PROGRAM ELEMENT NO. ACSL1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS HEALTH EFFECTS RESEARCH LABORATORY OFFICE OF RESEARCH AND DEVELOPMENT U.S. ENVIRONMENTAL PROTECTION AGENCY RESEARCH TRIANGLE PARK, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Neurophysiological methods fall into two broad categories, macrophysiological and microphysiological. The unit of analysis for microphysiological methods is cellular or subcellular (e.g. membrane or channel) whereas the unit of analysis for macrophysiological methods is a population of neurons. Each set of methods may be used to great advantage or disadvantage, depending upon the experimental question at hand. The purpose of this chapter is to focus on studies which have used macrophysiological methods to investigate the neurotoxicity produced by organometals. For each study to be discussed, the issues under consideration will be (1) appropriateness of the method to answer the experimental question; (2) adequacy of experimental design in using the method; (3) importance of the findings; and (4) identification of unresolved issues.

Only a few organometallic compounds have been investigated using macrophysiological techniques, and it will become evident that these investigations have been incomplete. A future goal of this chapter is to stimulate interest in pursuing these studies, so that we might achieve a more complete understanding of the compounds and the methods we use to study them.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED 20. SECURITY CLASS (This page) UNCLASSIFIED	21. NO. OF PAGES 110 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Macrophysiological Assessment of Organometal Neurotoxicity

by

Robert S. Dyer
Neurophysiology Branch
Neurotoxicology Division
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Contents

I. Introduction....2
II. Macrophysiological Methods
III. Organomercurials....4
A. Methylmercury....4
1. General....4
2. Clinical Reports....5
3. Characterization of Toxicity....7
a) EEG....7
b) sensory....10
c) excitability....18
d) perinatal toxicity
e) peripheral nervous system toxicity....20
B. Other Organomercurials....21
IV. Organotins....22
A. Triethyltin (TET)....22
1.General....22
2.Clinical Reports....23
3.Characterization of Toxicity....23
a) EEG....23
b) visual system....25
c) neuronal excitability....29
d) perinatal toxicity....34
e) peripheral nervous system toxicity....38
4.TET as a Model Compound....39
a) degenerative/demyelinating disorders...39
b) edema and demyelination....43
B. Trimethyltin....45
1.General....45
2.Characterization of Toxicity....47
a) EEG....47
b) sensory systems....48
c) neuronal excitability....53
C. Other Organotins....61
1. EEG Studies....61
2. Neuronal Excitability Studies....62
V. Other organometals....62
VI. Summary and Conclusions....64
A. Methylmercury....64
B. Triethyltin....65
C. Trimethyltin....66
D. General....67
VII. Acknowledgements....68
VIII. References....69
IX. Figure Captions....80

Introduction

Neurophysiological methods fall into two broad categories, macrophysiological and microphysiological. The unit of analysis for microphysiological methods is cellular or subcellular (e.g. membrane or channel), whereas the unit of analysis for macrophysiological methods is a population of neurons. Each set of methods may be used to great advantage or disadvantage, depending upon the experimental question at hand. The purpose of this chapter is to focus on studies which have used macrophysiological methods to investigate the neurotoxicity produced by organometals. For each study to be discussed, the issues under consideration will be (1) appropriateness of the method to answer the experimental question; (2) adequacy of experimental design in using the method; (3) importance of the findings; and (4) identification of unresolved issues.

Only a few organometallic compounds have been investigated using macrophysiological techniques, and it will become evident that these investigations have been incomplete. A further goal of this chapter is to stimulate interest in pursuing these studies, so that we might achieve a more complete understanding of the compounds and the methods we use to study them.

Macrophysiological Methods

Studies involving several macrophysiological methods will be considered. To provide a framework for interpreting these studies, it is important to know some general characteristics of the methods. For example, do we know what the method measures? Can the method be used to detect or characterize neurotoxicity? Can the method be used

to assess mechanisms of neurotoxicity? Does the method have an analog in human studies which would allow straightforward extrapolation?

The methods selected for coverage in this review were EEG, evoked potential, multiple unit, kindling, afterdischarge seizures, drug induced seizures, and maximal electroshock seizures. Unfortunately, no relevant multiple unit studies were found. EEG and evoked potential studies are of interest, because the methods are easily applied to both human and nonhuman species. Studies involving various models of seizures are interesting because they provide some measure of the excitability of the brain.

Interpretation of EEG studies must always be tempered with an understanding of the behavioral state of the subject, since in most cases these covary. For example, in the relaxed wakeful condition, there is a higher incidence of alpha (8-12 cps) activity than in other normal states. Depending upon the species and recording site, a high incidence of theta activity (4-8 cps) might reflect either sleep or locomotor activity. In recordings from the rat hippocampal formation, the frequency of theta correlates well with the vigor of activity as well (e.g. Whishaw and Vanderwolf, 1983). Therefore, a finding that a particular organometal produced increased theta could only be understood in the context of knowing whether recordings were made in unrestrained rats and whether those rats were more or less active than controls. High quality quantitative studies of EEG are difficult to perform, and few have been done. It is unlikely that this method will ever be of much use in assessing mechanisms of toxicity, but it may well become useful for detecting and characterizing neurotoxicity. It should be considered

largely untested at this point.

Evoked potential methods assess the responsiveness of a particular system to a known stimulus. Used prudently, they can detect and help characterize neurotoxicity. Their ability to determine mechanisms of toxicity is extremely limited, but they may be useful in helping to determine the direction which mechanism-oriented studies should take.

Models of seizure activity in the brain differ in the means by which seizures are elicited, and therefore differ in the specific nature of excitability tested. In some cases there is detailed knowledge of the brain regions or systems activated by the eliciting event, while in others there is not. Unless several models of seizure activity are utilized in a particular investigation, it is difficult to make accurate statements about the general excitability of the brain.

Most of the work to be summarized below has used the above described methods as aids to characterization of the nature of neurotoxicity. In a few cases, related measurements have been obtained in humans, and these will be described separately as clinical studies.

Organomercurials

A. Methylmercury

1. General

The human methylmercury poisoning incident at Minimata Bay, Japan, probably accounts for the most extensive neurotoxicity data base on any toxicant, and one of the most compelling cases for the heightened vulnerability of younger subjects. Among the many symptoms of methylmercury poisoning are sleep disturbances, changes in motor func-

tion, cognitive function and sensory function. Somatosensory and visual systems appear to be particularly affected in humans. As has been pointed out by Merigan et al. (1983), the visual system deficits are relatively unique since they appear to result largely from cortical damage, rather than more peripheral effects. Further, the cortical damage seems to occur primarily in the depths of the sulci within the visual cortex, thereby raising questions about the suitability of experimental models of the dysfunction in species which are lissencephalic. While there is an extensive series of behavioral and neuropathological studies which have been performed upon animals following exposure to methylmercury, most neurophysiological studies which have been performed have involved either peripheral nerve studies or studies which are too molecular/micro in nature to fall within the realm of this review. There are relatively few studies which focus upon macroelectrode recordings from the CNS.

2. Clinical

A number of studies have reported the effects of exposure to methylmercury on EEGs in humans. Typical among these is a systematic study of human EEG alterations by Brenner and Snyder (1980), who reported a descriptive longitudinal evaluation of the EEG from a family of 10 in New Mexico, 4 of whom suffered from methylmercury poisoning. The individual from whom the most extensive recordings were made (14 recordings in all) received prenatal exposure to methyl mercury, and at 9 years of age was blind, had spastic quadriparesis, and myoclonic jerks, but could evidently hear loud noises. The nature of the EEG analysis appears to have been visual inspection of the records. During the first two months of life three recordings all appeared normal,

but since then all recordings have shown marked abnormalities. The abnormalities have included bilateral multifocal spikes, with occasional generalized spike and polyspike discharges. In addition, background EEG rhythms showed diffuse slowing and excessive high amplitude beta waves, a pattern indicative of severe underlying brain damage. The extent of the EEG abnormalities correlated well with the age and overt neurological manifestations of the subjects, such that the mother, who had no EEG abnormalities, also had no other neurological symptoms. Perhaps the most interesting finding from this study is that no epileptiform spikes were observed in the recordings from subjects who never showed clear evidence of behavioral seizures. One might have expected that if severely affected subjects had seizures, then mildly affected subjects might have epileptiform abnormalities in the EEG in the absence of seizures, but such was not the case. Thus, the visual scoring of EEG records added nothing to the neurological exam. Computer analysis of the EEGs might have been more sensitive.

Sensory system disturbances are prominent among complaints of individuals exposed to methylmercury. Mukuno et al.(1981) compared behavioral and electrophysiological methods for evaluating visual function in 12 humans with Minimata disease of 15-25 yrs duration. The data were contrasted to those obtained from 7 healthy physicians. The behavioral tests included visual acuity, the Arden grating test (a combination of acuity and contrast sensitivity) and visual field evaluation. The electrophysiological test was a pattern reversal evoked potential (PREP), in which vertical black and white bars with 50% contrast and spatial frequencies from 0.5-8.0 cycles/degree were reversed at an unspecified rate. The authors measured latency of the

first positive peak and peak-to-peak amplitude between the first positive and second negative peaks. The peak latency tended to increase in the exposed subjects, but the most useful dependent variable was based upon the rate of amplitude reduction produced by increasing spatial frequency. From this variable, the authors calculated the spatial frequency at which the amplitude was 50% of what it was at 0.5 cycles/degree. There was a very high correlation between this value and the score obtained by subjects on the Arden grating test (r=.85). While the Arden test is clearly simpler to administer, the authors point out that it is subject to psychogenic factors. The outcome of the PREP test is not easily altered by motivational or other related factors. Further, this test is readily applied to nonhuman species.

Snyder and Seelinger (1976) evaluated the integrity of the peripheral nerves in the individuals tested by Brenner and Snyder (1980) and described above. While the affected individuals reported somatic sensory disturbances, there were no alterations in measures of sensory nerve conduction. The authors conclude that the sensory effects must not be peripheral in nature, supporting a similar conclusion reached by Von Burg and Rustam (1974), who studied patients involved in the most recent Iraq epidemic (Bakir et al., 1973). However Cinca et al. (1979) have shown that in two cases of ethyl mercury poisoning there was a clear and reversible slowing of conduction in sensory nerves.

3. Characterization of Toxicity

a) Effects of methylmercury on the EEG - Sleep disturbances (both hypersomnia and insomnia) have been reported in humans exposed to

methylmercury, but no detailed or quantitative assessment of these disturbances has been reported. Using EEG recordings, Arito et al. (1983) evaluated the sleep/wakefulness cycle in rats exposed to 3 different dosages of methylmercury. The purpose of the study was presumably to determine whether rats also exhibited sleep disturbances following exposure to methylmercury, and if so to characterize the nature of the disturbance.

Arito et al. (1983) present EEG data obtained from 16 audlt Sprague-Dawley rats with chronic electrodes implanted in the skull over the frontal and occipital cortex, and in the dorsal neck muscle for recording the EMG. The rats were divided into 3 groups, each of which received a different dosage of methylmercury (1.65 mg/kg/day for 2 days, 5.0 mg/kg/day for 2 days, or 15 mg/kg/day for 2 days). Unfortunately, there were no animals that received only vehicle treatment. After a 3 day period of habituation to the test chamber, EEGs were recorded continuously for 4 consecutive test days prior to administration of the methylmercury, and on selected test days after administration. On test days, each minute of EEG was scored visually and classified as either W (wakeful), SWS (slow wave sleep) or PS (paradoxical sleep), for the full 24 hours. Statistical treatment of the data consisted of t tests, and therefore must be considered to reflect a very nonconservative analysis. Despite this nonconservative approach, no effects were reported for the low dose group.

The authors report data which indicates two types of effects. Only in animals receiving the highest dosage of methylmercury was the total amount of time in which they were either asleep or awake altered. On the first full day following treatment, these animals spent 69% of

their time in either SWS or PS, whereas during the baseline (pre treatment) period animals spent about 54% of their time in sleep. The next test day reported for this group was 12 days after the end of treatment, and at this point total sleep time had reverted to baseline levels (55%). In contrast to the total amount of sleep and waking time, the relative distribution of sleep and waking EEGs occurring during the light and dark phases of the day were altered at lower dosages and for longer periods. In general, this effect was characterized by a transient dampening of the day/night rhythm for W and SWS states, and a more persistent phase shift for the PS state. In the high dosage group the phase shift was still evident 2 months after exposure. These results are summarized in Fig 1. In the intermediate dosage group, there was a slight shift in the amount of waking EEG activity from the dark to the light, and a slight shift in the amount of SWS from the light to the dark.

The findings indicate a clear disruption in the sleep/wake pattern in rats. To determine the brain levels of mercury related to athese effects, the authors performed regional brain dissertions and analyzed the tissue with atomic absorption spectrophotometry. The high dosage level produced peak brain levels of 10 ug Hg/g wet whole brain on day 10, and the intermediate dosage produced peak levels of about 2 ug Hg/g. If the data from the intermediate dosage group are truly statistically significant (which they may not be, based upon the type of analysis reported), then the sleep disturbance produced by methylmercury occurs at very low levels compared to other effects

(Arito et al., 1983). In addition to these findings, the authors state that spectral analysis of the background EEG revealed no differences between exposed and treated rats, although these analyses were done only on 2 rats, and details were not reported.

Mattsson et al. (1981) included a spectral analysis of the EEG of 3 beagles exposed to methylmercury as young adults as part of what was primarily an evoked potential study (see below). In contrast to the lissencephalic brains of rats, beagles have an extensive pattern of sulci. Therefore, this species may provide a better model of the CNS effects of methylmercury than the rat. The dogs were exposed daily to 0.5 mg/kg methylmercury chloride p.o. for the duration of the experiment, and recordings, which began about 3 weeks before the daily exposures were initiated, occurred within daily 20 min test sessions. Test sessions continued for up to 2 months after the onset of treatment. It is not clear from the paper exactly when, with respect to the evoked potential studies, the EEGs were collected. However the authors indicate that no EEG changes occurred until "toward the end of the exposure period," at which point the amount of high frequency (20-30 Hz) activity in the EEG nearly doubled. These data were not discussed, and conflict with the findings in rats (no change in EEG spectra) and humans (increased slow waves) described above.

b) Effects of methylmercury on sensory systems - As mentioned previously, sensory system effects are among those most frequently reported following exposure to methylmercury. Using the experimental protocol described above, Mattsson et al. (1981) also recorded flash evoked potentials (FEPs) from 3 adult beagles, before and following exposure to methylmercury. In an effort to minimize alterations in the FEPs

produced by alterations in attention and arousal, the authors trained the dogs to perform a cued avoidance response. FEPs were obtained during performance of this task.

This was an entirely within subjects design, such that each dog's post exposure waveforms were compared to preexposure waveforms. The analysis was unconventional in the neurotoxicology literature. Evoked potentials may be regarded from two opposite perspectives. Viewed from one perspective they may be regarded as waveforms containing a series of unique, defined peaks at relatively consistent poststimulus latencies, each with a characteristic underlying generator and with different significance for the system under study. When viewed from this perspective, each evoked potential peak is evaluated independently of others, with respect to its latency from the stimulus and its amplitude. The presumption is that a specific peak may be affected by the treatment under study, and that identifying the peak affected and the nature of the change provides insight into the nature of toxicity. Alternatively, the evoked potential may be regarded as a complex waveform which should be analyzed as a whole. The advantages of this pattern approach over the peak analysis approach are (1) that a single derived number may be used to describe an evoked potential or a difference between evoked potentials, thereby simplifying statistics, (2) that little prior knowledge about the generators, etc. of the specific evoked potential studied is required, and finally, (3) by not throwing away all of the data points that do not occur at peaks, more information from the recorded response is taken into consideration when comparisons between waveforms are made. The latter approach may be useful for screening, but it precludes obtaining systematic information about the nature of effect. While the former

approach is most common in neurotoxicology, and in evoked potential research in general, Mattsson et al. (1981) elected to use the latter approach.

Using the pattern analysis approach, Mattsson et al. (1981) detected alterations in the FEPs of the 3 dogs about 1 week after the onset of treatment. In a parallel series of dogs, it was determined that brain levels of mercury in the occipital cortex were about 1.28 ug/g at this time. The authors detected no alterations in performance of the cued avoidance task, nor were there any other obvious behavioral changes among the dogs. Clinically obvious poisoning did not occur until nearly 2 months after the onset of treatment. Therefore these data appear to support the argument that FEPs may be useful for screening for early effects on sensory systems. However they provide little insight into the nature of the visual dysfunction produced in dogs at these early exposure points. Fig 2. summarizes these findings.

Since the damage to the visual system following exposure to methylmercury appears to be primarily cortical in nature and related to the pattern of gyri and sulci, it is not at all clear that visual dysfunction should be expected in the lissencephalic rat. Nevertheless, two studies have examined the impact of perinatal exposure to methylmercury upon visual function using evoked potential techniques.

Zenick (1976) recorded FEPs from 30 day old male rats which had received one of five basic exposure conditions, using cross fostering to evaluate carry over between conditions. One group was never exposed

to methylmercury. The other groups received exposure either throughout gestation and nursing, during nursing only, during gestation only, or during the postweaning period. The postweaning group received 2.5 mg Hg/kg/day "directly", presumably by i.p. injection, for postnatal days 21-30. Other exposures were indirect, through the mothers, who received approximately 2.5 mg/kg/day in their drinking water.

Only 5 rats were evaluated in each treatment condition, and each rat tested was randomly selected from a different litter. All recordings were made under chloralose anesthesia. The author did not describe any efforts to control body temperature, which can be expected to vary under anesthesia, and variations in which can be assumed to produce large effects on latency of evoked potentials. Amplitudes were not evaluated, because the author found them too variable. The results of this study are difficult to interpret for several reasons. First, while the representative waveform presented by the author for FEPs recorded from the rat visual cortex appears similar to those reported by others, the peak designations are reversed in terms of which peaks are called positive and which are called negative. Thus, the first peak in most FEP recordings is P1, followed by N1, but by the author's labeling scheme N1 precedes P1. One must either infer that the peaks were mislabeled or that the waveform was aberrant. For purposes of discussion, it will be assumed that the peaks were mislabeled since the only technical problem noted was an unduly restricted bandpass (6-600 Hz).

The author indicates that in the control rats the P1 (his N1) was 68 msec, and that the N1 (his P1) latency was about 123 msec. These are extraordinarily long latencies. For comparison, unanesthetized

rats usually have P1 and N1 latencies of 20-25 and 28-32 msec, respectively (e.g. Dyer et al., 1978). Anesthetized rats have P1 and N1 latencies of 24-26 and 33-35 msec, respectively, and deeply hypothermic (body temperature about 31C) anesthetized rats have P1 and N1 latencies of about 35-37 and 46-48 msec, respectively (Dyer and Boyes, 1983; Hetzler and Dyer, 1984). The effect reported by the authors was that exposure to methylmercury shortened P1 latencies to about 45 msec and N1 latencies to about 72 msec. While these are enormous latency changes, they still fail to bring the waveforms within normal range. Therefore, despite the care which the author took to include appropriate controls during the exposures, some unidentified factor in the recording procedure makes the data difficult to interpret.

A different approach to exposure and recording was taken by Dyer et al. (1978). A single dosage of either corn oil or 5 mg/kg methylmercury was given to pregnant rats on day 7 of gestation. On postnatal day 65, 18 control and 13 exposed female rats (from 6 and 4 litters, respectively) were implanted with chronic electrodes for recording FEPs. FEPs were obtained at 4 different flash intensities, and peak latencies as well as peak-to-peak amplitudes were measured and analyzed. Increasing flash intensity normally increases peak amplitudes, but the rats treated with methylmercury exhibited greater increases in amplitudes of the early peaks (P1N1 and N1P2) than controls. In addition, latencies of the P2 and N2 peaks were shorter in the exposed group than in the control group, independent of flash intensity. The authors interpreted the amplitude effect in terms of a general "brain damage" model, which is still plausible (Dyer, 1986). The latency decreases were attributed to a selective loss of slowly

conducting fibers, since methylmercury does appear to produce selective toxicity to small cells. It is important to note that the latency decreases were about 2 msec for P2, and about 5 msec for N2, and that these changes were about an order of magnitude less than those reported by Zenick (1976).

While both Zenick (1976) and Dyer et al. (1978) report altered FEPs in rats following treatment with methylmercury, it is not clear whether these findings reflect specific visual system deficits or general brain dysfunction. Other studies have suggested general brain dysfunction (e.g. learning and memory deficits) in similarly treated rats (Eccles and Annau, 1982; Zenick, 1974) but no studies have specifically addressed the possibility that visual deficits exist in these rats, nor has this possibility been confirmed neuropathologically.

The extent to which exposure to methylmercury produces auditory dysfunction is in some question. Some clinical reports indicate no auditory dysfunction (e.g. Brenner and Snyder, 1980), while others have described sensorineural hearing loss in affected individuals (e.g. Nosaka et al., 1970; Friberg, 1971). The clinical reports which describe auditory dysfunction suggest that it is cortical in nature, although there is no compelling evidence to support this contention. From controlled laboratory studies it is evident that methylmercury can accumulate in the guinea pig cochlea (Konishi and Hamrick, 1979), and that auditory dysfunction can be detected in adult rats given 50 mg/kg methylmercury and tested using the acoustic startle test (Wu et al., 1985). These findings suggest that the dysfunction may be peripheral or subcortical.

Recently Wassick and Yonovitz (1985) used the BAER to test mice

exposed to methylmercury as adults. Two exposure groups were used, and two sets of recordings were made in each group. In one group (n=5), mice received 4 mg/kg s.c. methylmercury daily for 17 consecutive days. In the other group (n=6) the mice received 8 mg/kg s.c. methylmercury for 6 consecutive days. While the first group received more total methylmercury (68 mg/kg), the second group's total exposure of 48 mg/kg was more concentrated. Recordings were obtained at 4 times: prior to treatment, and on days 7, 14 and 28 after the onset of treatment. There was no control group, and no statistical analysis of the data was reported.

The first set of recordings performed by Wassick and Yonovitz (1985) was intended to determine physiologically the auditory threshold of the rats at different pure tone frequencies. To do this, BAERs were recorded to pure tones of descending intensity, until the experimenter could no longer visualize a BAER in the tracing. The procedure was repeated over a wide range of pure tone frequencies (4-78 KHz) to characterize any selective changes. The second set of recordings consisted of BAERs obtained at 80 dB SPL intensity at each of the frequencies tested previously. In this set of recordings the latency of BAER peaks was measured. This procedure was presumed to allow localization of dysfunction produced by methylmercury.

While the data were presented primarily in graphic and tabular form with no statistical analyses, it appears that exposure to methylmercury increased the threshold for producing BAERs. Fig 3 summarizes the effects of methylmercury on BAER thresholds. While the posttreatment data points are clearly shifted with respect to the pretreatment points, it would have been useful to see a control group which showed no such shift with time, particularly in view of the ap-

parent variability between the two sets of pretreatment recordings. In addition, a question of some theoretical interest is whether the shift in threshold was greater at some frequencies than others. While the authors conclude that there was a preferentially greater shift at the frequencies of 16 and 32 KHz for the 4 mg/kg/day group, it is impossible to determine whether these are valid conclusions in the absence of statistics.

The results of the peak latency analysis are even more difficult to interpret, since data are presented from only one mouse in each group. However the data presented suggest that at least in the 8 mg/kg group the effect of treatment was to shorten all peak latencies. These data were considered consistent with those reported by Zenick (1976) and Dyer et al. (1978) in the visual system.

A plot of the interpeak latencies (Peak IV-Peak I), an indicator of central rather than peripheral dysfunction, is given for both treatment groups at the frequency of greatest hearing sensitivity, 16 KHz, in Fig 4. These data seem to indicate a decreased central conduction time, implying increased conduction velocity through the brainstem. The mechanism by which this might occur is not clear.

From these studies it is evident that methylmercury produces auditory dysfunction in mice, and that this dysfunction contains

peripheral and brainstem components. The extent to which dysfunction in the auditory cortex of mice occurs remains to be investigated. Preliminary evidence based upon exposure of adult squirrel monkeys to methylmercury suggests that there may be significant species differences in the nature of auditory dysfunction. However these findings, presented in abstract form, provide too little detail for evaluation (Zook and Wilpizeski, 1980).

c) Effects of methylmercury on neuronal excitability - As with many neurotoxic compounds, poisoning with methylmercury can lead to seizures. A presumption implicit in the work of a number of investigators, and the validity of which remains to be determined, is that clues to the nature of toxicity produced by substances which trigger seizures at high dosages may be obtained by studying neuronal excitability following administration of lower (nonconvulsant) dosages. Several such studies have been performed with methylmercury.

Eccles et al. (1981) investigated the influence of prenatal exposure (gestational day 15) to methylmercury chloride (5 mg/kg by gavage to the mother) or the corn oil vehicle upon hippocampal afterdischarges recorded during adulthood. The exposed animals were not different from controls on variables specific to the afterdischarge itself (e.g. threshold and duration), however two measures of postictal excitability were affected. Normal hippocampal afterdischarges end in a profound EEG depression. The end of the EEG depression is usually signalled by what has been called the rebound afterdischarge (Dyer et al., 1979). In the exposed rats, the time from the end of the afterdischarge to the beginning of the rebound was longer, implying an increased period of hypoexcitability. In addi-

tion to the EEG depression, afterdischarges are followed by a longer lasting period during which it is more difficult to elicit a subsequent afterdischarge. This period was nearly three times as long in the methylmercury exposed rats as it was in the controls. The functional implications of this finding are not clear.

Su and Okita (1976) took a different approach to studying long term changes in excitability produced by prenatal exposure to methylmercury. In this study methylmercury hydroxide was given to pregnant mice subcutaneously on gestational day 10, in dosages of 0, 6, 8, or 12 mg/kg. The mice were tested on postnatal day 70 with flurothyl, inhalation of which is known to produce behavioral convulsions. In experiments which use flurothyl, the dependent variable is usually the time required for convulsions to appear once the animals are placed within the exposure chamber. In this study, the highest prenatal dosage of methyl mercury produced an increase in sensitivity to these convulsions, as measured by decreased latency (threshold) to exhibit maximal seizures. This test produced a different answer than the afterdischarge test described by Eccles et al. (1981), since it suggests increased excitability where the afterdischarge test suggested no change in seizure threshold, but decreased postictal excitability.

Menashi et al. (1982) evaluated audiogenic seizure susceptibility in 28-31 day old mice exposed prenatally to methyl mercury. In this study 8 mg/kg methyl mercury chloride was administered subcutaneously to pregnant mice on gestational day 12. The audiogenic seizure test involves placing the animal next to a loud bell or buzzer, turning on the bell and observing whether or not a seizure occurs. While the

difference between the percentage of control and treated mice exhibiting seizures was small (18% of the control mice exhibited seizures, compared to 33% of the treated mice), the large number of mice used (189 in all) rendered the difference statistically significant. Thus, this study too seems to show a slight but general increase in the excitability of the brains of mice treated prenatally with methylmercury.

While it is tempting to assume that the differences between the results of Eccles et al. (1981) and those of Menahsi et al. (1982) and Su and Okita (1976) reflect differences in the tests, the difference in species and exposure paradigms should not be ignored. One must conclude that the issue of persistent changes in excitability produced by methylmercury is still unresolved.

d) Effects of methylmercury on the peripheral nervous system - From the human data it was unclear to what extent the sensory dysfunction was produced by peripheral effects, and to what extent it was produced by central effects. Murai et al. (1982) reported an experiment designed to resolve whether sensory deficits produced by methylmercury poisoning were central or peripheral in nature. Peripheral nerve conduction was studied in rats (sural nerve was stimulated, recordings were made from the sciatic). The rats were administered 5 mg/kg of methyl mercuric chloride every other day for either 32 or 54 days. The amplitude and latency of the nerve response were recorded. Sensory evoked potential data recorded from 2 Macaque monkeys who received 5 mg/kg methylmercury chloride orally once/week for 10 weeks were described. Visual evoked potentials, somatosensory evoked potentials and brainstem auditory evoked potentials were all recorded, but no details of the recording procedures

were described. All recordings were made under "mild anesthesia induced by ketamine hydrochloride." Sensory and motor conduction properties were studied in the median and sural nerves of both monkeys.

In rats, exposure for 54 days abolished recordings from the sural nerves. Recordings from animals treated for 32 days exhibited reduced amplitude of recordings, but did not exhibit appreciable slowing of conduction. In the monkeys there were no signs of peripheral nerve involvement, although the sural nerve amplitude measurements were evidently so variable as to preclude meaningful comparisons. Both somatosensory and visual evoked potentials showed progressive slowing, while the brainstem auditory evoked potentials were unaffected. The authors concluded that only very severe poisoning produces peripheral involvement. The work of Cinca et al. (1979) suggests that even in the case of severe poisoning in humans, the peripheral nerve effects are transient. It may also be that in rats peripheral nerve involvement is demonstrated more readily than in other species (Miyami et al., 1983). B. Other organomercurials

While other organomercurials are used as fungicides, there are few studies which have systematically investigated any of them using electrophysiological approaches. It therefore remains to be determined whether the observations by Cinca et al. (1979),that ethyl mercury poisoning in humans produces peripheral sensory loss and demyelination of the ninth and tenth cranial nerves, are true of methylmercury also, or are peculiar to ethyl mercury.

Organotins

Of all the organometallic compounds, organotins have received the most attention from neurophysiologists using macrophysiological techniques. In particular, triethyltin and trimethyltin have received considerable attention. In both cases the primary direction of research appears to be from the perspective of these compounds as prototypes for investigating different kinds of neurotoxicity with different methods.

A. Triethyltin

1. General

Triethyltin (TET) is a compound which achieved some attention in the 1950's, when it was discovered to be the contaminant of Stalinon responsible for over 100 deaths in France. TET proved to have some very interesting properties. Acute administration of TET produces intramyelinic edema, splitting of the myelin and vacuolation. In addition, it appears to uncouple oxidative phosphorylation. A potent action of TET which was not revealed until relatively recently is that it produces marked changes in thermoregulation and thermoregulatory behavior. Rats injected with 6 mg/kg TET and maintained in normal ambient conditions (e.g. 22C), may have a reduction in rectal temperature as great as 5C. Many of the acute effects of TET (physiological and other) which have been described in the literature may in fact be secondary to hypothermia. As will be seen below, some physiological studies have attempted to evaluate the role of hypothermia in TET neurotoxicity, but with the exception of a study designed to study behavioral thermoregulation (Gordon et al., 1984), no behavioral studies have addressed this confounding variable.

2. Clinical Reports

Among the first electrophysiological observations following exposure to TET were those reported in abstract form by Pruill and Rompel (1970). These authors recommended that clinicians interested in making the differential diagnosis of alkyltin neurotoxicity in humans would benefit from inclusion of an EEG examination. To support this recommendation, the authors describe features common to 5 cases of "acute organo-tin intoxications" in young persons employed in a chemical laboratory. Unfortunately, the only indication from the abstract that the organotin in question was TET, if indeed there was only one organotin, is the mention of Stalinon in the preceding paragraph. This abstract never seems to have become a full length article, so the details will remain obscure. However the abstract was provocative because it indicated that such exposures could lead to symptoms of pseudotumour cerebri with irritation. Visual defects were noted, as was the appearance of a sharp wave (seizure?) focus over the occipital lobes. The authors suggest that while hypoxia might account for these symptoms, edema probably cannot. While it may be unreasonable to be critical of details omitted from an abstract, it is lamentable that no full length paper followed.

3. Characterization of Neurotoxic Effect

a) Effects of TET on the EEG-Benedek et al. (1976) have evaluated the influence of a single injection of TET upon the EEG of unanesthetized cats with chronic electrodes over the frontal, temporal and parietal cortex. Two weeks after the implantation surgery, the cats (numbers unspecified) were treated with either 1.0, 2.5, 5.0 or 10.0 mg/kg TET sulfate. EEGs were recorded over the first 6 hrs, and then daily for

up to 13 days following injection. Analog filters were used to divide the signals into frequency bands of 1-4 cps (delta), 4-8 cps (theta), 8-13 cps (alpha) and 13-30 cps (beta).

The overt behavioral consequences of the injection could be divided into three phases: an initial acute toxicity phase lasting 6-8 hrs, a phase of apparent recovery, lasting 24-72 hrs, and a return of the manifestations of toxicity (e.g. muscle weakness). A similar pattern has been well described for rats exposed to acute TET (e.g. Torack et al., 1970). Cats treated with the 10 mg/kg dosage did not survive the acute toxicity phase, while those treated with 5 mg/kg died about 30 hrs after treatment. Changes in the EEG paralleled the overt behavioral manifestations of toxicity. The 1 mg/kg TET treatment did not alter the EEG. An injection of 2.5 mg/kg TET produced EEG alterations which were evident within 15 min, as evidenced by an increased synchronization and the appearance of slow waves (3-4 cps). The appearance of slow waves was particularly apparent in the recordings from occipital cortex. By the third hour, the occipital slow waves were nearly continuous. At 6 hrs the incidence of slow waves had decreased substantially. However beginning 24 hrs after treatment there was a return of slow waves over the occipital cortex, which increased over the next several days. From the fifth day on, the occurrence of slow waves again decreased, but even on the 13th day there was still evidence of abnormal slow wave activity over the visual cortex.

These findings, despite the absence of statistical evaluation of the data, are interesting. First because the EEG abnormalities parallel the behavioral ones, and secondly because the EEG abnormalities

are the ones to be expected from white matter lesions (e.g. Gloor et al., 1977). It would have been useful to know the status of the white matter during the different phases of recording. As the authors point out, the initial slow waves occurred too early to be considered a result of edema. It is not clear whether TET-induced hypothermia may have contributed to this finding. The authors do not indicate the ambient temperature at which tests were performed, nor do they indicate whether body temperature was monitored. Presumably it was not. Perhaps the most significant finding of this study was the apparent sensitivity of the visual (occipital) cortex to the effects of TET. The basis for this apparent sensitivity was not discussed.

b) Effects of TET on the Visual System - The clinical EEG report of Pruill and Rompel(1970), the experimental EEG investigation of Benedek et al. (1976), and reports of visual disturbances in exposed humans (e.g. Barnes and Stoner, 1959) have implicated the visual system as an early indicator of TET neurotoxicity. In addition, neuropathological studies have suggested special involvement of the visual system in the neurotoxicity produced by acute administration of TET (e.g. Scheinberg et al., 1966).

Dyer and Howell (1982a) evaluated the integrity of the visual system in TET-exposed rats, using flash evoked potentials (FEPs). Long-Evans hooded rats were fitted with chronic skull electrodes a week prior to the onset of testing. Pretreatment recordings were obtained from 38 rats. The rats were subsequently divided into groups for daily treatment with either saline, or 0.188, 0.375, 0.75 or 1.5 mg/kg TET bromide in saline. These treatments, administered 2 hrs before testing, continued for 6 consecutive days. FEPs were recorded on

each treatment day, and for 2 days following termination of treatment.

TET produced evidence of visual dysfunction, as indicated by increased FEP latencies. There are several interesting features of these data. While FEP latencies were affected in general, the latency to peak N3 was most often affected. N3 latencies were increased at the lowest dosage of TET (0.188 mg/kg/day), while other peaks were only affected at the highest dosage of TET used (1.5 mg/kg/day). Effects of TET on amplitudes were inconsisent and difficult to interpret. There was a considerable lag between the onset of treatment and when latency alterations began to appear (several days), however the increased latencies remained for up to several weeks after termination of treatment. Fig 5 shows the gradual disintegration of one waveform over days with treatment, and Fig 6 shows the temporal characteristics of the change in N1 latency.

While these data do not demonstrate the nature of visual system dysfunction produced by TET, they do indicate that the cortex may be at least as sensitive as the optic nerve, since N3 latencies were affected at dosages below those required to affect latencies of peaks P1 and N1. The increased latencies observed are consistent with what would be expected in the case of disturbances in myelin, but similar changes could occur as a result of hypothermia. The study also fails to demonstrate any special sensitivity of the visual system. Concur-

rent tests of hippocampal function (see below) indicated that this system is at least as sensitive as the visual system to the neurotoxicity produced by TET.

In a related study, Dyer and Howell (1982b) investigated the impact of hypothermia upon FEPs recorded from TET-treated rats. In this study, rats were divided into 4 groups. Two groups received 6 mg/kg TET bromide in a single injection, and one of these two groups was maintained in a warm (30°C) environment for 7 hrs. The remaining two groups received saline, and one of these was also maintained in a warm environment for 7 hrs. FEP recordings were obtained immediately before treatment, and 1.5, 4, 7, 24 and 120 hrs post treatment. The warm environment was presumed, based upon another study, to reduce TET-induced hypothermia to a minimum.

As expected, TET produced increased latencies. However contrary to expectation, prevention of hypothermia appeared to exacerbate the toxic consequences of exposure. Whereas the rats treated and maintained at normal room temperatures had normal FEPs 24 hrs following treatment, the rats in whom hypothermia was prevented still had abnormal FEPs 120 hrs later. Furthermore, since even the warmed animals had increased latency, the authors suggested that previous results (Dyer and Howell, 1982a) could not be accounted for simply on the basis of hypothermia. The differences between the two treated groups and their respective control groups are represented in Fig 7.

These studies neither substantiate any unique susceptibility of

the visual system, nor do they indicate the nature of visual dysfunction produced by TET, however they do indicate that monitoring the FEP may provide a marker as to the magnitude of an acute TET-induced encephalopathy.

A further step towards characterizing the effects of acute exposure to TET upon the visual system was taken by Boyes and Dyer (1983). In this study, pattern reversal evoked potentials (PREPs) were recorded in addition to FEPs, with a purpose of comparing the relative sensitivity and specificity of the two techniques. While FEP studies are technically simpler to perform than PREP studies, and for this reason are probably better adapted for screening purposes, alterations in PREPs are more easily interpretted. Specifically, under the appropriate circumstances PREPs may be used to describe such complex visual functions as acuity and contrast sensitivity (e.g. Boyes et.al, 1985).

Boyes and Dyer (1983) recorded from chronically implanted Long-Evans hooded rats immediately before and 24 hrs after treatment with either 0, 4.5 or 6.0 mg/kg TET bromide. During the interval between treatment and testing, the rats were maintained in a warm (30°C) environment, to prevent hypothermia. The results indicated that as was the case with FEPs (Dyer and Howell, 1982b), TET produced latency increases in PREPs 24 hrs after a 6 mg/kg treatment. Further, in contrast to FEPs, TET produced a pattern of amplitude alterations in PREPs which consisted primarily of increased amplitudes in the early components. While the significance of this finding for visual function is not clear, it does suggest that TET produced more than a disruption in myelin. Alterations in evoked potential amplitude are usually taken to reflect the synchrony, magnitude and nature of synaptic events (Dyer, 1986a).

(c) Effects of TET on Neuronal Excitability - As part of their effort to characterize the effects of TET, Dyer and Howell (1982a) included an evaluation of hippocampal afterdischarges. There were several reasons for this test. It was considered important to evaluate a system other than the visual system, to determine whether unique sensitivities existed. The limbic system, and in particular the hippocampus was studied because it is known to be disrupted by exposure to a number of toxicants. In particular hippocampal function is altered by hypoxia, and histotoxic hypoxia is one of the consequences of TET exposure (Aldridge and Rose, 1969). The hippocampal afterdischarge was presumed to tap disordered hippocampal function, because an AD should require the complex interplay of excitation and inhibition in order to produce the rhythmic pattern of spikes and relative electrical silence (Dyer et al., 1979).

In this study three ADs were produced each day, and from these a total of 11 variables was obtained on each day. The variables included measures of the threshold for producing the AD, the duration of the AD, spike frequency, magnitude and pattern of the postictal EEG depression, and postictal electrical excitability. The results demonstrated a mixed excitatory/inhibitory effect of TET, depending upon the dose and the endpoint. The low dosage of TET (0.188 mg/kg/day) decreased AD thresholds relative to control, while higher dosages did not. All but the lowest dosage of TET increased the frequency of spikes within the AD, and the 0.375 mg/kg/day dosage produced a greater postictal electrical excitability than the other dosages. All of these effects are arguably a function of increased excitability. On

the other hand the high dosage (1.5 mg/kg/day) produced a decrease in postictal electrical activity, and postictal electrical excitability. Both of these effects are clearly indicative of decreased neuronal excitability. By virtue of its greater ease of interpretability, this is the more compelling observation. It is interesting to note that there was not a significant dose x day interaction described for the analysis, suggesting that the effects were of constant magnitude across days. This implies that in some cases a single treatment with a dosage as low as 0.188 mg/kg TET might alter hippocampal function. Fig 8 illustrates the influence of different dosages of TET upon postictal electrical activity.

The minimum conclusion from these findings is that the visual system does not afford unique sensitivity, since TET-induced effects were observed in the AD at the same dosages. In addition, TET produced evidence of CNS depression. Whether there was a biphasic effect of TET (excitation and depression) cannot be determined until the biological determinants of the different AD variables is understood more fully.

Several studies by Fox and Doctor support the presumption that TET produces a depressant effect upon the CNS. In these studies the MES (Maximal Electroshock Seizure) model was used to evaluate excitability. MES Seizures are produced by application of an AC current pulse either to the scalp, the ears or to the corneas, and the timing and sequence of resulting behaviors is recorded. The model has the

advantage that it is rapid and does not require the use of surgical or pharmacological intervention. The model has the disadvantage that a large percentage of apparently normal rats (20-35%) do not show the "typical" pattern (e.g. Fox and Doctor, 1983). Also, despite the extensive use of this model in the testing of anticonvulsant compounds, it is not entirely certain exactly what is being tested. While early workers in the area attempted to standardize electrode placements, recommending the corneas (Woodbury and Davenport, 1952), no such standardization has occurred. Recently Browning and Nelson (1984) have compared the seizures elicited by corneal and ear-clip electrodes, and found them to be substantially different from each other. The studies by Doctor and Fox used placements which were on the scalp between the eyes and the ears.

The seizures described by Fox and Doctor (1983) were graded on a 5 point scale of increasing severity. Grade 1 consisted of clonus, grade 2 was tonic forelimb flexion, grade 3 was tonic forelimb flexion followed by forelimb extension; grade 4 consisted of tonic forelimb flexion and hindlimb flexion followed by forelimb extension; and grade 5 seizures were characterized by tonic forelimb flexion and hindlimb flexion followed by forelimb extension and hindlimb extension. In addition to scoring the seizures, Fox and Doctor (1983) also recorded the duration of each phase. In some studies (e.g. Fox, 1982) extension/flexion seizure duration ratios were calculated for the hindlimbs and forelimbs. Under these circumstances, more severe seizures were manifested by larger values of the ratio, and anticonvulsant activity was reflected by smaller values.

In female Long-Evans rats (Fox and Doctor, 1983), acute treatment

with TET bromide (1 and 5 mg/kg) produced a decreased seizure severity compared to vehicle injected controls. The decreased severity was manifested by a lower seizure grade, by decreased forelimb extension durations and increased forelimb flexion durations (i.e. decreased extension/flexion ratios). It is interesting that when the seizures were severe enough for both hindlimb flexion and extension to occur, hindlimb extension/flexion duration ratios did not appear to be affected by the treatment. Perhaps most important about these findings however is that the effect of a single injection of TET was effective in reducing seizure severity for several days.

The same paper reports a separate experiment in which smaller quantities of TET (0, 1, 5 and 10 ppm) were placed in the drinking water for 10 days. The data presented were collected on days 1, 2 and 10 of exposure, and on days 1, 6 and 14 after exposure. The 1 ppm dose produced a daily intake of about 1.25 mg/kg, and reduced seizure grade scores by the second day of exposure. Scores were still reduced 1 day after exposure, but not 6 or 14 days after exposure, and there were no alterations in flexion or extension durations. The 5 ppm dose produced alterations on the first day of exposure, and these were still evident 14 days after exposure was terminated (Fox and Doctor, 1983). These data are summarized in Fig. 9.

While this paper presents a wealth of data, the analysis of the data is confusing. For example, the authors state that in the subacute experiment, rats were tested daily during exposure and daily for the

first week after exposure, providing a minimum of 17 tests per rat. Unfortunately only data from a few days are presented. With this experimental design a repeated measures analysis of variance is appropriate so that dose x time interactions may be assessed. While the authors indicate that they performed an "overall unweighted means analysis of variance," they neither specify the nature of the analysis or its outcome. Are the data they present highly selected or are they representative? Were the analyses performed upon all data or only those reported? The reader has no way of knowing.

As part of an effort to compare the neurotoxicity of several trialkyl tin compounds, Doctor and Fox (1982) used the MES test in mice injected i.p. with either 3.5×10^{-6} or 17.5×10^{-6} mol/kg of TET bromide. The low dose produced no significant effects on seizure grade, but the high dose produced an anticonvulsant effect similar to the one reported in rats. Similarly, seizure durations were affected by TET treatment. In this case the low dose increased the extension/flexion ratios at 0.5 and 4 hr time points, but not the 22 and 96 hr time points. The high dose was effective also at the 22 hr time point, but not at 96 hrs. Again, the details of the analysis of variance were not made available.

The sensitivity of the MES test, and the long duration of the depressant effect produced by TET are both impressive. It is not at all clear how this effect might come about. Possibilities raised by the authors include alterations in the chloride concentration gradient and a change in the pH equilibrium (Doctor and Fox, 1982; Fox and Doctor, 1983), or some change in either GABA or alpha adrenergic neurotransmitter systems (Fox, 1982). While some evidence exists to

support either view (e.g. Selwyn et al., 1970; Selwyn, 1976; Costa, 1985), neither argument is persuasive, partly because the organotins most effective at producing the relevant changes in biochemical parameters are least effective as anticonvulsants (Selwyn et al., 1970; Costa, 1985; Doctor and Fox, 1982), and vice versa. A change in the impedence of the brain might also produce an anticonvulsant effect, and such changes have been noted following administration of TET (Shelton et al., 1982), but the dosages required to produce impedence changes were probably too high to explain the MES findings. Thus, the answer to this puzzle remains to be discovered.

d) Perinatal toxicity produced by TET - Only two studies have assessed the physiological consequences of perinatal exposure of rats to TET. Dyer et al. (1981) gave i.p. injections of TET bromide to rat pups on postnatal day 5. A within litter design was used, such that 1 male and 1 female from each litter received 0, 3, 6 or 9 mg/kg TET, and each litter contained all treatments in both genders. Electrodes were implanted when the rats were 60 days old, and testing began 10 days later.

An extensive series of tests was performed, over the course of about 1 month. On the first test day FEPs were recorded, followed by hippocampal afterdischarges. Beginning the next day, 17 consecutive days of kindling stimulation was presented to the hippocampus. Three to six days later, pentylenetetrazol-induced seizures were evaluated, and six to nine days later, picrotoxin-induced seizures were evaluated.

Despite the extensive evaluation of neuronal excitability, there was only trivial evidence of any long term alteration produced by TET.

Female rats given 3 mg/kg TET on postnatal day 5 had slightly longer cortical afterdischarges during the hippocampal kindling process than did controls. No other changes in afterdischarge parameters, kindling parameters, pentylenetetrazol seizures or picrotoxin seizures were significant. The non-dose related nature of the AD finding, coupled with its relative isolation in the face of many presumably related endpoints which were unaffected by exposure, suggest that this may be a type I error.

In contrast to the lack of any long term effect of perinatal TET on neuronal excitability, Dyer et al. (1981) found considerable effects on the FEP. In particular, there was a progressive dose dependent latency increase in successive peaks, as indicated in Fig 10. The fact that the early FEP peaks were unaffected by the treatment suggests that the enduring effect was cortical in nature, and could be accounted for by assuming disturbed myelination in the visual cortex.

More recently, Doctor and Fox (1983) reported a study of the immediate and long term effects of perinatal exposure to TET on MESs. In this study rats were treated i.p. with either 0, 0.25, 0.5, 1.0, 2.5 or 5.0 mg/kg TET bromide on postnatal days 0, 5, 10, 15 and 20. MES testing occurred on days 10, 12, 14, 16, 18, 20, 23, 26, 36, 46, 56, and 85. The authors report that TET produced a developmental delay in appearance of the normal MES pattern, but that when the rats became adults their seizure severity was greater if they had been exposed to TET during the early postnatal period. Fig 11 illustrates the seizure severity scores from this study. These findings appear non-

dose related. However extrapolation to the age at which each group reaches 60% (the approximate "normal" percentage of stage 5 MESs in rats), indicates a very clear TET-induced developmental delay. Fig 12 illustrates the duration of hindlimb extension at different ages. Increased duration implies increased severity. While the authors suggest that treatment produced a biphasic effect (retarded development of the response, coupled with an increased severity once it developed) an alternative explanation may be more parsimonious. Note that control animals had their longest durations on day 22, and that this peak was followed by a progressive 20% decline in duration. Treated groups tended to peak later and decline less thereby supporting the initial explanation of a developmental delay.

There are problems with the analyses presented in this study. The seizure duration data were collected from the same animals, over and over, on the different test days. The authors indicate that each rat was tested on each of the twelve different test days, which would make the design of this study a simple groups x days repeated measures analysis. However the authors then indicate that 205 rats were studied between days 10 and 26, 101 rats, all females, were studied between days 36 and 56, and that 53 females were studied on day 85, including none from the 0.25 mg/kg group. The authors state that the

analysis performed was an unweighted means ANOVA, but no further details were given regarding (a) how the repeated testing nature of the analysis was taken into consideration, particularly in the face of grossly decreasing numbers with increasing time; or (b) what the outcome of the analyses were. Further, since the authors have previously indicated that typically up to 35% of adult rats do not show the normal seizure pattern, it seems curious that all of the controls did show such a pattern on day 85 (see Fig 11). The controls appear to be the outliers on this test day.

Analysis of Fig 11 and Fig 12 suggests that very little change in the nature of the effect occurs between day 26 and day 85. It is therefore difficult to argue that the effects observed during adulthood reflect some sort of reorganization or compensation. The authors point out that a similar pattern of effect (depression initially, followed by increased activity) is observed when locomotor activity is used as the endpoint, following a single administration of TET on postnatal day 5. However in the latter case the increased activity observed in adulthood is considerably more impressive (Reiter et al., 1981). It is also interesting to note that in the case of the behavioral studies, the effect was greater in males than females, while only females were tested in the Doctor and Fox (1983) paper.

From these developmental studies one may conclude that perinatal exposure to TET produces both immediate and persistent effects. The immediate effects have only been evaluated using the MES test, and seem to be similar to the immediate effects observed in adults administered TET. The effects which persist into adulthood have been evaluated with a number of tests. Persistent changes in excitability

appear to reflect delayed development at unspecified loci, while persistent changes in the visual system are indicative of cortical dysfunction.

e) Peripheral nervous system toxicity - Using the rare but refreshing combination of electrophysiological, morphological and biochemical techniques, Graham et al. (1976) evaluated the consequences of exposure to TET sulfate in the drinking water (20 mg/liter) upon the peripheral nervous system of Osborne Mendel rats. Assuming a daily intake of about 30 ml/rat, the daily dosage of TET to these 250 gm rats was about 2.4 mg/kg. This dosage was sufficient to produce paralysis by 20 days, and the authors were required to take pains to avoid mortality (they were only partially successful).

The sciatic nerve was studied (each nerve was studied only once/rat), and the endpoints recorded were: electrical threshold for producing a compound muscle action potential; amplitude of the evoked muscle action potential; and motor conduction velocity. Repeated t tests were performed upon the data, which was collected on days 0, 10 and 20 of exposure, and at various times after exposure was terminated. No alterations were observed in the threshold for production of the response, or in the amplitude of the muscle action potential. The results of the conduction velocity test are illustrated in Fig 13. The figure illustrates a decline in conduction velocity, which was reversible over time.

The decline in conduction velocity was correlated with the ap-

pearance of splitting and vacuole formation within the myelin sheath of the sciatic nerve. There was also an increase in the number of neurofilaments in the axons of both vacuolated and nonvacuolated fibers, but this was not correlated with the alteration in conduction velocity since motor nerve conduction velocity "returned to normal at a time when florid changes were still present in the sciatic nerves during the recovery period (Graham et al., 1976)."

It is important to note that while significant alterations were observed in the peripheral nerves of these rats, the dosage of TET required to produce the alterations was substantial compared to the dosages described above as sufficient to produce alterations in measures of CNS function. This comparison suggests that the CNS is more sensitive to TET than is the PNS.

4. TET as a Model Compound

While few would argue that TET is a compound which poses significant environmental risk, it has nevertheless been used extensively in the laboratory as a model compound. Part of the reason for its attractiveness is most surely that it produces dramatic but reversible effts.

a) Degenerative/demyelinating disorders - Gerren et al. (1976) combined neurophysiological, biochemical and behavioral endpoints in an investigation of the effects of TET on mice. The stated purpose of the study was to assess the feasibility of using TET as a model of nervous system demyelinating diseases. Among issues the authors addressed were: (1) the nature of functional disturbances produced by acute and chronic exposure to TET: (2) the extent to which the time course of functional disturbances paralleled the known morphological

disturbances produced by TET; (3) the extent to which functional disturbances were central or peripheral in origin. Presumably a good model of a disease like multiple sclerosis (MS) would show some functional alterations of CNS origin similar to those produced by MS. These functional alterations should be correlated with structural alterations, which should be restricted to the CNS. Ideally the model would also demonstrate the episodic nature of MS, characterized by dysfunction, remission and dysfunction.

Gerren et al. (1976) examined the integrity of the peripheral nervous system by using sciatic nerve conduction velocity and threshold for eliciting a compound action potential in the sciatic nerve. Both of these endpoints were obtained from mice anesthetized with chloral hydrate.

In the chronic study, 6 mice were injected i.p. daily for 27 days with 2 mg/kg TET in 30% ETOH and 6 others were injected with the vehicle. The authors reported that 24 hrs after the last injection there was a 20% increase in the threshold for producing a peripheral nerve response, but that once responses were initiated they were conducted more rapidly (by about 5%) in treated mice. No statistics were reported, and it is therefore difficult to evaluate the significance of these findings.

Some mice (numbers not reported, so presumably small numbers), were tested about an hr after a single i.p. injection of 8 mg/kg TET in 30% ETOH. In this acute test, the authors reported a 75% increase in threshold for eliciting a response, and 10-15% increase in conduction velocity in the TET animals compared to control. In the absence of knowing the numbers of subjects involved, the relationship between nerve temperature and each group's values (we are only told

temperature was controlled between 36-37C), or what if any statistical evaluation was performed, it is difficult to place much confidence in these findings. It is interesting that the acute and chronic effects were similar, since the acute effects occurred prior to a time when much in the way of morphological alteration should be expected, leaving inhibition of oxydative phosphorylation as the most likely culprit. However before these findings are taken seriously they should be repeated with a more rigorous experimental design, particularly since they conflict with those of Graham et al. (1976).

To evaluate the integrity of the CNS, the authors recorded flash evoked potentials and EEGs from the visual cortex in unanesthetized mice with previously implanted electrodes. Only acute exposure to 8 mg/kg TET was studied, and recordings were made .03, .5, 1, 1.5, 2 and 24 hrs after treatment. Again, it is not specified how many animals were involved in the treated and control groups. The authors report that TET produced a shift in EEG power to lower frequencies. These findings are summarized in Fig 14. Again no indication is given as to how these effects were evaluated statistically.

Figure 14 indicates that in control and treated mice the peak activity is in the 5-8 Hz or theta range. It has been demonstrated that in rats, theta dominates during locomotor activity, and that the frequency of theta is directly correlated with the vigor of activity (Whishaw and Vanderwolf, 1973). Therefore Figure 14 suggests that the treated rats were simply less vigorous in their activity than controls.

The authors' description of the behavior of these mice as lethargic supports this suggestion. Since the chronic treatment with TET produced mice which were hypoactive when left alone, but which were made hyperactive in response to handling, it would have been interesting to record their EEG under both conditions. If the EEG effect was secondary to the behavioral effect, then the chronic animals should have had a lower dominant frequency than controls when at rest, but a higher dominant frequency than controls when handled. In such a case the EEG would have added nothing to behavioral observation. On the other hand, if the treated animals had a lower dominant frequency than controls independent of locomotor activity, the two would be dissociated. Unfortunately the authors did not record from any of the mice in the chronic exposure group. The authors made no attempt to relate the EEG findings to those produced by demyelinating disease, and it is therefore not clear why the measure was included.

Flash evoked potentials were recorded in the same mice from whom EEGs were obtained. No details of the stimulating, recording or analysis strategies were included in the paper. However the authors reported that in treated mice, amplitude of the "earliest response component was elevated and the next component depressed" compared to controls. All peak latencies were increased. Fig 15 illustrates the time course of the latency change for the "first peak." The effect appeared greatest at 2 hrs following injection, at which time there was about a 10 msec, or 30% increase in peak latency.

Superficially the evoked potential findings appear to support the

thesis that TET may be used as a model for MS, since patients with MS show delayed sensory evoked potentials as well. However the likelihood that in this case the TET effect was secondary to hypothermia clouds such an interpretation. Based upon the data of Gordon et al. (1984), 8 mg/kg TET should have produced a drop in body temperature of about 11° 1 hr after injection at an ambient temperature of 20°C. Such a drop in body temperature could easily produce a latency shift of the magnitude reported here, and might also account for amplitude alterations (Dyer and Boyes, 1983; Hetzler and Dyer, 1984). The authors concluded that TET may be useful for testing models of MS etiology. However based upon the above analysis, the value of TET as a model for degenerative disorders is questionable.

b) edema and demyelination-TET does not produce demyelination in the classical sense. However the edema and myelin splitting produced by TET may amount to a partial functional demyelination. Shah et al. (1978) and Amochaev et al.(1979) have used this property of TET to investigate the influence of disordered myelin upon the auditory system. In particular, the authors were interested in determining the relationship between TET-induced changes in myelin and latency of peaks in the brainstem auditory evoked response (BAER) of Sprague-Dawley rats. Their presumption was that if a strong correlation existed between BAER latencies and myelin content, then BAERs could be used as a noninvasive measure of myelin defects. In addition, the authors evaluated the relative effects of TET upon the peripheral and central auditory system by including a measure of central conduction time. Since the first BAER peak (peak I) reflects activity in the periphery, while subsequent peaks reflect activity in the CNS, the difference in latency between the first

and later peaks (known as central conduction time) provides an index of alterations which are in addition to any produced in the periphery and simply passed along.

Amochaev et al. (1979) used an exposure schedule identical to the one used by Graham et al. (1976), and described above. Briefly, the rats were exposed to 20 mg/L TET sulfate in their drinking water for 2 weeks. BAERs were recorded from needle electrodes placed in the scalp while the rats were anesthetized with α-chloralose. The stimuli were clicks presented at 60 dBHL above the experimenters' threshold. No efforts to control body temperature were described. Further, the records were highly filtered (100 Hz to 3KHz), which distorted the waveform compared to unfiltered recordings (Janssen et al., 1986). However if one can assume that the authors did control body temperature, then their findings indicate that TET produced an increase in those BAER latencies which reflect activity in both the peripheral and central nervous system. Further, the authors were able to show that these changes were reversed if the rats were tested 2 weeks after termination of exposure. Myelin content, determined in the same aniamls, paralleled the alterations in BAER latencies. Myelin content was reduced by about 50% at the end of exposure, but had recovered in the animals tested 2 weeks later. It is important to note that while these findings demonstrate TET-induced dysfunction in the auditory system, they tell us nothing about what the consequences of exposure to TET are in terms of hearing. Altered latencies to click stimuli carry with them only the presumption of disturbed hearing.

B. Trimethltin

1. General

Interest in trimethyltin (TMT) blossomed with the description by Brown et al. (1979) of the intriguing pathology produced by the compound. A sequence of neuronal cell loss which appeared to target certain cell fields within the hippocampus made for attractive comparisons between TMT and the contemporary neurobiological tool kainic acid. Further, the difference in the pathology produced by TMT and TET, coupled with the apparent structural similarity between the two compounds reinforced our inability to understand structure/activity relationships.

Like TET, TMT is not presently a compound of great environmental significance. While it has been reported (Hallas et al., 1982) that microorganisms in the Chesapeake Bay can biomethylate inorganic tin, and that picomole concentrations of TMT have been found in a variety of river and estuarine locations (Byrd and Andreae, 1982), it is not clear that the levels reached have biological significance. TMT is a byproduct of the manufacture of other organotin compounds, and as such may present an occupational hazard. The only known cases of human exposure to TMT come from occupational accidents (Fortemps et al., 1978; Ross et al., 1981). In these reports, exposed humans reported a variety of symptoms, including seizures which persisted to 6 months after termination of exposure, EEG abnormalities (low voltage spikes), lowered threshold to pentylenetetrazol seizures, aggressive behavior, irritability, insomnia, forgetfulness and dimming of vision.

In the case of TMT, the neuropathological and behavioral observations of exposed animals, and the clinical observations in exposed hu-

mans have guided the physiological studies. The neuropathology produced by TMT has been most extensively described in the limbic system. In the hippocampal formation, damage appears to be species and to some extent strain dependent, a factor which must be kept in mind when performing and interpreting physiological (and other) studies. The major differences in pathology seem to be between rats and mice. In mice, the primary damage is to the granule cells of the dentate gyrus, while in rats the primary damage is to the hippocampal pyramidal cells (Chang et al., 1983). Which pyramidal cells, and which granule cells are affected appears to depend upon the location along the septotemporal axis in Long-Evans rats, although other strains have not been investigated (Chang and Dyer, 1985). Gerbils appeared to be mouse-like, in that damage was more extensive in the granule cells of the dentate gyrus than in the pyramidal cells of ammons horn, while hamsters appear to have even less granule cell damage than rats. Marmosets showed more diffuse damage than has been reported for most species, including damage in the retina, lateral geniculate and visual cortex (Brown et al., 1984).

Aggressive behavior, whether towards conspecifics or towards attempts at handling by the experimenter, has been described following administration of TMT in Wistar rats (Porton strain) (Brown et al., 1979), Long-Evans hooded rats Dyer et al., 1982), and Marmosets (Brown et al., 1984), but not in Fisher rats (Bushnell, personal communication), Wistar rats (Royalhart strain?) (Loullis et al., 1985), Hamsters (Brown et al., 1984), gerbils (Brown et al., 1984), C57BL/6N or BALB/c mice (Wenger et al., 1984), grasshopper mice (Hulebak, personal communication), or primates (Bushnell, personal communication).

Self-mutilation of the tail has been described in Long-Evans rats and Sprague Dawley rats (Dyer et al., 1982; Sloviter et al., 1985) but not in other strains (Loullis et al., 1985). Seizures have been reported by many authors, although they evidently do not occur in all species or strains tested (e.g. Dyer et al., 1982; Brown et al., 1984).

2. Characterization of Toxicity

Most physiological studies of TMT toxicity have been designed to further characterize the toxicity, rather than to determine its mechanism. Most of these studies have focussed either upon sensory systems or upon the issue of CNS excitability. There is very little data on the effects of TMT upon the EEG in animals, which is particularly remarkable since insomnia and EEG abnormalities have been described in humans (Fortemps et al., 1978).

a) EEG - Ray (1981) has recorded the EEG from rats following exposure to TMT. In this study, rats with chronic electrodes at 5 cortical and 2 subcortical sites were studied. The subcortical sites consisted of a monopolar recording from the hilar region of fascia dentata and a bipolar recording between this dentate electrode and an electrode in the pyriform cortex. EEG recordings were made immediately before administration of the TMT (8-10 mg/kg by gavage), and at varying times over the next 11 days. The dosage of TMT employed was approximately the LD50 in the author's hands. The analysis of the EEG consisted of visual inspection of records, coupled with peak to peak measurements of 50 randomly selected 1 sec epochs on each test day.

Since the hippocampus is the primary generator of EEG activity in the theta range (4-8 Hz), and since the hippocampus is also one of the

structures with the most profound TMT-induced pathological alterations, it is not surprising that the only EEG effects reported by Ray (1981) were derived from the hippocampal recordings. Hippocampal theta has been closely tied to movement (e.g. Whishaw and Vanderwolf, 1973), and the hyperactivity produced by TMT should therefore increase the occasions with which one sees theta activity in EEG records. On the other hand, since TMT produces loss of hippocampal neurons, it might be expected that either amplitude or occurrence of theta might decline.

Ray (1981) reported that both the amplitude and occurrence of theta increased between days 2 and 4 following treatment. While this period of increased theta corresponded with increased behavioral activity, the author states that the two were partly dissociated, since long periods of theta were present, even when the rat was immobile. Subsequently, the frequency of the waves within the theta episodes decreased, and after day 8 there were no longer any theta episodes noted in the recordings.

These findings are interesting, but do not provide much insight into the nature of TMT toxicity. With careful recordings from different loci in the hippocampal formation, more sophisticated analysis of the EEG, and more systematic monitoring of behavioral activities during recording sessions, this type of study might provide some insight into the nature of hippocampal theta, since the generators of theta within the hippocampus are still a subject of investigation.

b) sensory sytems - Information upon which one might predict the presence or absence of visual dysfunction in TMT toxicity was initially contradictory. While the clinical reports indicated dimming of

vision (Fortemps et al., 1978), the initial pathological report in rats sought but failed to find retinal pathology (Brown et al., 1979). Based upon the observation that TMT-treated rats often ran into walls in an open field, and that some of their other behaviors could be accounted for on the basis of visual system dysfunction, Dyer et al. (1982) evaluated the visual system.

Long-Evans rats with chronic electrodes had FEPs recorded from the visual cortex on days 1, 4, 8 and 16 following administration (by gavage) of either 0, 5, 6 or 7 mg/kg TMT chloride. The findings, illustrated in Fig 16, show that TMT produced significant alterations in this response. Specifically, latency of the first positive peak was increased at all dosages, and latency of the second positive peak was reduced in a dosage dependent manner. The amplitude of the first peak was also reduced. There was no dosage x time interaction, suggesting that the effect of treatment was immediate and unchanged over the course of the study. The pattern of amplitude and latency findings was consistent with a decreased input to the visual cortex, an explanation which was assessed by recording FEPs from the optic tract in another group of rats (Dyer et al., 1982a). As would be expected from a peripheral lesion, optic tract latencies were increased by exposure to 4, 5 and 6 mg/kg TMT. Since the magnitude of latency increase was roughly equivalent to the magnitude of increase observed in the cortex, the authors concluded that the full extent of the lesion occurred distal to the recording electrodes, in the retina. As with the cortically recorded FEP, the optic tract response was decreased in amplitude. The most parsimonious explanation of these findings, that a decreased output occurred from the retina, was supported by patho-

logical examinations showing a loss of retinal ganglion cells (Chang and Dyer, 1983). TMT-induced retinal lesions have subsequently been described by others as well (Bouldin et al., 1984; Brown et al., 1984). Fig 16 summarizes the FEP findings following TMT.

The presence of an easily recognizable, albeit different from control, FEP in TMT treated Long-Evans rats indicates that while they have visual system dysfunction, they are not blind. A detailed description of this visual impairment is still lacking.

The apparent irritability of some TMT-treated rats suggested to some investigators that TMT might produce an increase in the amplitude of the acoustic startle response. However quite to the contrary, these authors found a profound decrease in the amplitude of acoustic startle response (Howell et al., 1981). This observation, which has been replicated a number of times (e.g. Ruppert et al., 1985), suggested that TMT may produce dysfunction in the auditory system in addition to the visual system. A full accounting of the effects of TMT on the auditory system has not yet been produced. However some evidence indicates that TMT increases the stimulus intensity required to produce a brainstem auditory evoked response (BAER) (Janssen and Dyer, 1984), and further that there is cochlear pathology associated with exposure to TMT (Chang and Dyer, 1983). All of these changes evidently appear quite rapidly, and may be present within 24 hrs of treatment.

In contrast to the above findings, Ray (1981) has reported alterations in auditory evoked potentials of a different nature. Evoked potentials were recorded from the same cortical and hippocampal loci as described above under EEG studies. The analysis described is not a conventional one, in that peaks are not numbered and there is no "standard" waveform presented with markings to identify what the author analyzed. The potentials recorded appear to have had primary and secondary components. The author defines one primary component with a latency of 12 msec, and two secondary potentials, one which occurred with a peak at about 28 msec after the stimulus, and another with a peak at about 60 msec after the stimulus. Evidently nearly identical responses were recorded from cortical and hippocampal loci, with the exception that no primary response was noted in the hippocampus. The interesting feature of these recordings is that the author reports an increase in amplitude of the secondary responses on days 1 and 2 following oral administration of 8-10 mg/kg TMT, but no change in the primary response on these days. These changes occurred before the EEG changes noted in the same study (see above). By day 4, the increased amplitudes had returned to normal, and beyond day 4 both primary and secondary responses declined in amplitude. These findings are illustrated in Fig 17. As the author points out, the absence of any effect upon the primary response suggests that the alteration is not a reflection of damage to the peripheral auditory system, and thus the findings are at odds with those of others (Howell et al., 1981; Janssen and Dyer, 1984; Ruppert et al., 1985). Ray (1981) suggests that the increased amplitude reflects an increased excitability at some level of the CNS, although there is no reason to suppose

that this is either true or not true.

Howell et al. (1982) have used physiological methods to assess the likelihood that TMT also produces somatosensory dysfunction. The most compelling suggestions that this might be the case came from the self-mutilation observed in TMT-treated rats, and the fact that visual and auditory systems had been affected by TMT, as described above. To evaluate the somatosensory system, Howell et al. (1982) recorded somatosensory evoked potentials from rats with previously implanted chronic skull electrodes. The authors used electrical stimulation of the tail nerve to elicit the response, and evaluated the threshold and conduction velocity of this nerve in order to assess the role of peripheral factors should cortically recorded alterations be detected.

For a variety of reasons, the somatosensory evoked potential has not been well characterized in rats (Dyer, 1986b). It is therefore difficult to compare the data recorded by Howell et al. (1982) with other studies for purposes of assessing adequacy of technique. However the authors describe a waveform following stimulation of the tail nerve, which was significantly altered in rats treated with TMT. The primary effect of the treatment appeared to be an increase in latency, which could not be accounted for on the basis of an altered peripheral conduction velocity. Some latencies were increased 1 day after treatment, while others did not increase until the second post treatment test point which was at 4 days. The authors did not follow

the time course of these findings beyond 4 days. As with the other sensory systems, there may be anatomical correlates of this somatosensory dysfunction, since Chang and Dyer (1983) have reported alterations in dorsal root ganglion neurons following exposure to TMT.

Perhaps the most significant feature of this collection of studies is that they identified sensory systems as targets for TMT. Neurophysiological techniques have not been used effectively to further describe the nature of that toxicity, although in the case of the visual system the study identified the retina as the probable locus of dysfunction. More sophisticated use of neurophysiological techniques may be expected to provide further insight into the real consequences of exposure to TMT for the sensing organism.

c) neuronal excitability - There were at least three compelling reasons to suppose that TMT might produce a change in CNS excitability. First, humans and rats with sufficiently high exposure levels exhibited seizures. Second, humans showed a lower threshold for elicitation of pentylenetetrazol seizures (Fortemps et al., 1978). Finally, the finding that TMT produces hippocampal damage and aggressive behavior, coupled with reports that limbic system kindling produces aggressive behavior (Pinel et al., 1977), suggested that a kindling like process might be involved in the reported aggression. The effects of TMT on neuronal excitability have been approached from a number of physiological perspectives, and these different approaches have produced occasionally conflicting data.

Since TMT produces spontaneous seizures, it would be useful to have EEG recordings from different brain sites, so that the area of

the brain from which seizures were triggered might be identified. The closest any studies have come to such recordings is Ray's (1981). Ray implanted chronic cortical electrodes at several loci, and further included depth electrodes in the dentate gyrus and pyriform cortex. Of the 7 rats treated with 8-10 mg/kg TMT, 2 were in the process of having EEGs sampled when spontaneous seizures occurred. One of these events was on the 4th day following treatment, and the other was on the 8th post treatment day. The behavioral seizures described were similar to those which have been described in rats during limbic system kindling, consisting of rearing up on the hindlimbs and forelimb clonus. Because of this limbic-like seizure pattern, it is surprising that the only reported EEG changes during the seizure were from the cortical leads. Ray (1981) indicates that isolated cortical spike trains lasting 2-5 sec occurred in concert with the behavioral seizure. These discharges remained localized.

Dyer et al. (1982) attempted to characterize CNS excitability in TMT treated rats using a number of different approaches, including pentylenetetrazol (PTZ) seizure characteristics, hippocampal afterdischarge characteristics, hippocampal kindling rate and amygdaloid kindling rate. In the case of hippocampal afterdischarge characteristics, an attempt was made to correlate pathological data with the physiological findings. This battery of tests was selected to demonstrate whether any alterations in excitability observed were local or reflected general properties of the CNS.

To examine the effects of TMT upon PTZ seizures, Dyer et al. (1982) treated Long-Evans hooded rats with either 0, 5, 6 or 7 mg/kg

TMT chloride. Five days later, a time when many of the behavioral consequences of TMT administration in rats are most prevalent, the rats were administered either 30, 45 or 60 mg/kg PTZ, i.p. The authors report that TMT-treated rats had more severe seizures with shorter latencies to clonic seizure activity than did controls. In addition, the 7 mg/kg dosage of TMT reduced by nearly 1/2 the dosage of PTZ required to produce at least a clonic convulsion in 50% of the group. Browning (1985) has suggested that all but the tonic component of PTZ seizures is probably triggered rostral to the brainstem. These data are therefore compatible with alterations in excitability occurring in the forebrain, possibly in the limbic system. Fig 18 illustrates the effects of TMT upon PTZ seizures.

Hippocampal afterdischarges (ADs) are presumed to reflect the functional integrity of neurons and pathways within the hippocampus (Dyer et al., 1979). It was presumed that this measure would be extremely sensitive to TMT, since TMT produces dramatic changes in the morphology of the hippocampus. To investigate the influence of TMT upon hippocampal ADs, the authors implanted electrodes in the dorsal hippocampus of Long-Evans rats. Electrical stimulus intensity necessary to produce an AD (the AD threshold) was determined before treatment and on four post treatment test days: 1, 4, 8 and 16. The rats were treated with either 0, 5, 6 or 7 mg/kg TMT. On the four post treatment test days, rats were also stimulated at 4 times threshold AD current, to assess a variety of AD parameters. Following the 4 x

threshold AD test, post AD hypoexcitability was assessed by stimulating once every 5 min until a third AD appeared. While there was a trend towards increased AD thresholds over time in the TMT-treated groups, none of the AD parameters differed significantly from control. While this finding was quite unexpected, recent evidence suggests that hippocampal ADs, once triggered, may be controlled by nonsynaptic events (Traub et al., 1985). Therefore, changes in synaptic relations among cell groups within the hippocampus may not be well detected by this technique.

In addition to studying the properties of hippocampal ADs, Dyer et al. (1982) evaluated hippocampal excitability by studying the rate at which daily stimulation of the dorsal hippocampus produced kindling. In this study, rats were treated once with either 0, 4, 5 or 6 mg/kg TMT, and subsequently stimulated once/day for 30 days. The data indicated that rats treated with 6 mg/kg TMT kindled slightly (but significantly) more rapidly than did controls.

In a manner similar to the hippocampal kindling effect, Dyer et al. (1982) also demonstrated that TMT-treated rats exhibited more rapid kindling of the amygdala than did controls. These findings are summarized in Fig 19. In both the amygdaloid and hippocampal kindling studies the threshold for producing an AD was higher at the end of the experiment in TMT-treated than in control rats. The authors speculate that this may merely reflect cell loss produced by the treatment, a speculation which was supported by the finding of a correlation between cell loss and raised hippocampal AD thresholds.

The experiments by Dyer et al. (1982) make a clear case for increased neuronal excitability following TMT. The experiments have failed, however, to pinpoint the locus of the excitability change. Increased excitability may occur at a number of loci, and for a number of reasons. The AD data seem to indicate that excitability changes do not originate within the hippocampus, since TMT increased AD thresholds and did not change other AD parameters. However, as indicated above, these findings may reflect the nonsynaptic characteristics of ADs.

An additional study (Dyer and Boyes 1984), addressed the issue of hippocampal excitability following TMT somewhat differently. The authors reasoned that since the pattern of damage produced in the hippocampal formation following acute administration of TMT was similar to the pattern of damage produced by kainic acid, and since kainic acid damage is presumed to be partially mediated by increased activity in the mossy fiber system, secondary to reduced recurrent inhibition of granule cells (Sloviter and Damiano, 1981a; Sloviter and Damiano, 1981b), then TMT may produce a decrease in the recurrent inhibition of dentate granule cells.

Dyer and Boyes (1984) evaluated the status of recurrent inhibition in the dentate gyrus using the method described by Anderson et al. (1966). With this method, paired stimulations are presented to the perforant path, and the resulting responses are recorded from the dentate gyrus. Dentate gyrus responses to perforant path stimulation are generally characterized by a slow wave, which represents the EPSP generated by the input. If a sufficiently intense stimulus is presented, then a spike appears on the EPSP. This spike is a reflec-

tion of the synchronous action potentials of a large number of nearby granule cells, and is known as the population spike. The amplitude of the population spike is a reflection of the number of granule cells responding to the input. Since the first perforant path stimulus of a pair activates recurrent inhibitory pathways, the second stimulus, if close enough to the first in time, should produce a smaller population spike. The magnitude of decrease in the second population spike is a measure of the magnitude of recurrent inhibition, and the quantity is usually represented as a ratio [R2/R1].

Using unanesthetized rats, the authors first determined the magnitude of recurrent inhibition in all rats at an interstimulus interval of 20 msec. The rats were subsequently dosed orally with either 0, 5 or 6 mg/kg TMT chloride, and recurrent inhibition was evaluated at 2, 24 and 120 hrs post treatment. The findings which are summarized in Fig 20, indicate that TMT produced a very rapid change in recurrent inhibition. Within 2 hrs of exposure the effectiveness of the paired pulse in reducing the amplitude of the second population spike had declined by nearly 50%. These data support the contention that TMT does alter hippocampal excitability, and does so by reducing recurrent inhibition in the dentate gyrus. Subsequent morphological studies by Chang and Dyer (1985) have supported this interpretation by showing that dentate basket cells, the presumed morphological substrate of recurrent inhibition, exhibit degenerative changes shortly after treatment with TMT.

In addition to producing a local excitability change in the hippocampus, TMT also evidently produces alterations in the polysynaptic chain which, by way of the kindling process, allows localized ADs in the amygdala and hippocampus to become more generalized and ultimately trigger behavioral seizures. Further evidence that TMT produces complex alterations in polysynaptic systems comes from a study by Hasan et al. (1984).

Hasan et al. (1984) investigated the temporal course of TMT-induced changes in the limbic system of chronically implanted Sprague-Dawley rats. Two pathways were examined. Stimulating electrodes placed in the hilus of the dentate gyrus (DG) were used to elicit monosynaptic EPSPs from CA3 neurons. Stimulating electrodes located in the prepyriform cortex (PPC) were used to elicit polysynaptic EPSPs from DG neurons. The authors report that 7.5 mg/kg TMT produced a progressive reduction in the monosynaptic DG-CA3 response, beginning on the second day following treatment and reaching a minimum at about 14 days post treatment. In marked contrast, the same animals exhibited a transient but profound potentiation of the PPC-DG response which became evident on the third post treatment day and lasted until about the seventh posttreatment day. At this point the polysynaptic response began a progressive decline in amplitude, until the 30th day, when it was effectively absent. These results are summarized in Fig 21.

Based upon this study, it is not clear whether the potentiated PPC-DG response is an obligatory consequence of the polysynaptic nature of the test, whether there was something unique about either of the specific synapses involved, or whether the two specific synapses involved were unique. These questions may be answered directly with

similar studies using monosynaptic and polysynaptic recordings from the PPC-DG system. The study provides further evidence that TMT produces an increase in excitability of at least a segment of the limbic system.

Doctor et al. (1982) have addressed the question of TMT-induced changes in neuronal excitability using a variety of systemic convulsants. The experiments described by these authors differ from those described above in two important respects. First, the authors used mice, while the above described data on excitability were all obtained in rats. This is an important difference, since the neuropathology produced by TMT in the two species is substantially different (Chang et al., 1983). Second, the authors used dosages of 1.065, 2.130, 3.195 and 4.26 mg/kg TMT. While these would be considered relatively low dosages for a rat, the two high dosages used here are almost certainly lethal dosages for mice, where the LD50 is usually listed as between 2 and 3 mg/kg. The investigators performed tests of seizure susceptibility at 1 and 14 hrs following i.p. administration of the TMT. The convulsants used during the seizure susceptibility tests were bicuculline, pentylenetetrazol (PTZ) strychnine and isonicotinic acid hydrazide (NH), and were all administered in lethal dosages. The endpoints studied were: latency to clonic seizure, latency to tonic seizure and latency to death.

In general, effects of TMT were restricted to the 1 hr post treatment time period, and to the two high (lethal) dosages of TMT.

Under these conditions, TMT increased the latency to clonus (bicuculline and PTZ), tonus (INH) and death (bicuculline, INH, PTZ and strychnine). It does not seem appropriate to consider this short term effect of a lethal concentration of TMT as an anticonvulsant effect. No convulsions were prevented, and the slight increases in latency to different stages of convulsion could be attributed to metabolic alterations secondary to a profound acute and general debilitation. These findings should not, therefore, be considered in serious conflict with the above suggestion that TMT increases neuronal excitability.

On the other hand, Doctor and Fox (1982) investigated TMT-treated mice using the MES test described above. Using this test, the authors did appear to provide some evidence of CNS depression following TMT. Using a dosage of 3.5×10^{-6} mol/kg TMT, the seizure severity was not affected. However extension/flexion duration ratios were decreased, in a manner similar to what was reported for TET. This attenuated duration of the most severe aspects of the seizure lasted for about 24 hrs after treatment, which made it more effective than the equimolar dosage of TET. It is not clear whether this effect reflects acute debilitation of the mice, whether it reflects a unique property of alkyltins not evaluated by any of the other procedures for assessing neuronal excitability mentioned above, or whether it reflects the fundamental species difference in response to TMT.

C. Other Organotins

1. EEG studies

Mazaev et al. (1968) reported a study in which rabbits with chronic electrodes over frontal and occipital cortex were administered

either 25 mg/kg tetraethyltin or 100 mg/kg dichlorodibutyltin. Both of these dosages were lethal within 3-7 days. However the authors reported signs of EEG activation (low voltage, high frequency) immediately after treatment. No conclusions may be drawn from this observation.

2. Neuronal excitability studies

Using the MES test described above, Doctor and Fox (1982a, 1982b) have investigated a number of other organotin compounds. Tri-n-propyltin and tri-n-butyltin both produced a brief (0.5 - 4 hr) decline in the duration extension/flexion duration ratios. The effect was not as pronounced as for TET and TMT, and was only evident at a 17.5×10^{-6} mol/kg dosage.

A somewhat different pattern of effects was produced by tricyclohexyltin (TCT) and triphenyltin (TPhT). A dosage of 17.5×110^{-6} mol TCT/kg produced an increase in severity of the MES seizures, as indicated by increased duration of both forelimb and hindlimb extension. This effect was evident within 0.5 hr of treatment, and was still present at the last test time, 96 hrs after treatment.

TPhT, at a dosage of 17.5×10^{-6} mol/kg, produced first an increase in seizure severity (only evident 0.5 hrs after treatment), followed by a decrease in seizure severity (evident at 4 and 24 hrs after treatment), which was followed by recovery at the last test time of 96 hrs after treatment.

Other Organometals

While there have been a number of neurobehavioral studies of other organometals, virtually none of them have involved the use of

electrophysiological endpoints. One exception is work reported by Saito (1973), who recorded EEGs from rats exposed to tetraethyllead. In this study 12 adult rats with chronic cortical electrodes were injected i.p. with 1 ml gasoline without lead/100 gm body weight, and 9 rats were injected with leaded gasoline. The estimated dosage of lead was 16.5 mg/kg, which should have been near the LD50. EEGs were recorded 1 day before and on days 1-5, 7 and 10 after injection, while the animals were unrestrained and unanesthetized. Unfortunately there were no rats given an innocuous compound for purposes of control.

The EEG analysis performed consisted of feeding the signal into a 5 channel frequency analyzer, each channel of which passed only signals within either the delta (2-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta 1 (13-20 Hz) or beta 2 (20-30 Hz) bands. Energy within each of these bands was then integrated over time, to provide a quantitative estimate of activity. As noted previously, a disadvantage of EEG analyses in unrestrained animals is that meaningful interpretation can only be made in concert with contemporaneously recorded behavioral data. While Saito noted behavioral changes over days, no effort was made to rigorously correlate these changes with the EEG. The most striking findings in this study were that 7 days after administration of the gasoline containing lead, there was a great increase in the amount of alpha activity compared to the group receiving only gasoline, and 10 days after treatment the lead exposed group had a slightly higher theta and delta activity than the gasoline only group. These changes occurred at a time when rats were described as showing "excessive tension and excitement." However the signficiance of the findings is neither clear nor is it discussed.

Summary and Conclusions

Macrophysiological methods have been used to evaluate the neurotoxicity of only a few organometal compounds. In some cases the methods have failed to provide any interpretable information. In other cases, the methods have verified observations made using other approaches. A few studies have used these methods to identify previously unrecognized characteristics of neurotoxicity produced by the test compound.

A. Methylmercury

In the case of methylmercury, EEG studies verified in rats the sleep disturbances reported in humans (Arito et al., 1983). Evoked potential studies have verified visual dysfunction in dogs exposed as adults (Mattsson et al., 1981) and rats exposed perinatally (Zenick, 1976; Dyer et al, 1978), without shedding much light on the nature of dysfunction. The findings in rats were novel, since the rat brain is lissencephalic and since methylmercury is usually described as producing damage in the depths of sulci. It would be of interest to determine the extent to which methylmercury produced visual dysfunction in rats exposed as adults. However techniques more sophisticated than recording FEPs from the visual cortex will be required to characterize further this dysfunction. It is of interest to learn whether it is peripheral.

The BAER technique was used productively by Wassick and Yonovitz (1985) to demonstrate auditory dysfunction in mice exposed to methylmercury as adults. Auditory thresholds were raised by exposure. It is still not clear whether this is a frequency dependent phenomenon, whether it applies to other species, and the extent to which peripheral, brainstem and higher structures are involved. All of these issues could be resolved using macrophysiological approaches. There have been few physio-

logical studies of somatosensory function in animals exposed to methylmercury, although a thorough characterization of dysfunction should include such an evaluation.

Neuronal excitability has been evaluated in rats and mice following exposure to methylmercury. Perinatal exposure produces an increase in the period of hypoexcitability after a hippocampal afterdischarge (Eccles, et al., 1981). On the other hand, mice perinatally exposed to methylmercury demonstrate increased sensitivity to flurothyl and audiogenic seizures (Su and Okita, 1976; Menashi et al., 1982). Thus, while it appears that perinatal exposure to methylmercury alters neuronal excitability when tested later, the direction of the change may depend upon the species, age at dosing and test used. Future studies might profitably explore the relationships among different tests of neuronal excitability, because the case of methylmercury is not the only one in which the direction of effect may depend upon the test used.

B. Triethyltin

Attempts to use physiological methods to validate the use of TET as a model for studying degenerative/demyelinating disorders have been unconvincing, partly due to the failure to control for the hypothermic effects of TET (Gerren et al., 1976). Similar uncertainties cloud the interpretation of studies on the EEG (Benedek et al., 1976) and on auditory system (Amochaev et al., 1979).

Physiological methods have provided some support for the idea that the visual system is sensitive to TET, following acute exposure and also following perinatal exposure. Whether this sensitivity is unique among

sensory systems remains to be determined. TET also produces signs of depressed neuronal excitability, which when evaluated with the MES test are quite long lasting, even following acute administration (Fox and Doctor, 1983). There is as yet no clue to the mechanism of these effects.

C. Trimethyltin

Physiological methods have been particularly useful in characterizing the neurotoxicity produced by trimethyltin. In particular, evoked potential studies have identified the rat visual system as a target, indicating the probable locus as the retina (Dyer et al., 1982a). The BAER identified peripheral auditory dysfunction, and the SEP suggested somatosensory dysfunction. All of these findings were subsequently confirmed using pathological and/or behavioral data.

Evoked potential techniques have also been useful in suggesting that the nature of physiological dysfunction in the hippocaampus is in part loss of recurrent inhibition (Dyer and Boyes, 1984) and in part polysynaptic potentiation (Hasan et al., 1984). These techniques could be used to explore further the intriguing septotemporal distribution of damage produced by TMT in the hippocampus (Chang and Dyer, 1985).

Conflicting evidence exists regarding the influence of TMT on neuronal excitability. TMT increased rates of amygdaloid and hippocampal kindling, and increased sensitivity to pentylenetetrazol seizures in rats (Dyer et al., 1982c). On the other hand, Doctor and Fox (1982) reported that TMT depressed excitability in mice, as measured by the MES test. This effect was short lasting (about 24 hours), and may reflect either species differences in TMT toxicity or differences in what is measured by the test.

D. General

As the above summary indicates, evoked potentials have been particularly useful in providing initial characterization of neurotoxicity. However in all of these cases the methods were not used to full advantage. Considerably more could be learned from evoked potential tests about the sensory toxicity produced by organometal compounds, and this information could make easier the task of determining mechanisms of toxicity.

Studies of neuronal excitability have often provided conflicting information. While some of the conflict may reflect differences in species and experimental design, it is also clear that excitability is not a unitary phenomenon. Considerable work must be performed to determine what relationship (if any) exists between the many measures of neuronal excitability considered in this review.

The use of macrophysiological techniques has many limitations, most of which stem from difficulty in interpretation. However the preceding review should make it evident that there is much to be learned from these techniques, when properly applied. As future studies begin to unravel the meaning of the endpoints measured, the techniques should become progressively more valuable.

Acknowledgements

The author wishes to thank Greg Ridgon for reviewing the manuscript, Judy Smith for manuscript preparation and John Hoots for assistance with translation from German.

This manuscript has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

Aldridge, W.N. and Rose, M.S. (1969) The mechanism of oxidative phosphorylation. A hypothesis derived from studies of trimethyltin and triethyltin compounds. FEBS Lett., 4:61-68

Amochaev, A., Johnson, R.C., Salamy A. and Shah, S.N. (1979) Brain stem auditory evoked potentials and myelin changes in triethyltin-induced edema in young adult rats. Exper. Neurol., 66:629-635

Anderson, P., Holmquist, B. and Voorhoeve, P.E. (1966) Entorhinal activation of dentate granule cells. Acta Physiol. Scand. 66:488-460

Arito, H., Hara, N. and Torii, S. (1983) Effect of methylmercury chloride on sleep-waking rhythms in rats. Toxicol 28:335-345

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S. Dhahir, H.I., Clarkson, T.W., Smith J.C., and Doherty, R.A. (1973) Methylmercury poisoning in Iraq. Science, 181: 230-241

Barnes, J.M. and Stoner, H.B. (1959) The toxicology of tin compounds. Pharmac. Rev., 11: 211-231

Benedek, V.G., Szekeres, L., Zoltan, O.T. and Obal, F. (1976) Elektroenzephalographische untersuchung des experimentellen gehirnodems. Arzneim.-Forsch. 26, 335-340

Bouldin, T.W., Goines, N.G., and Krigman, M.R. (1984) Trimethyltin retinopathy. Relationship of subcellular response to neuronal subspecialization. J. Neuropathol. exp. Neurol., 43: 162-174

Boyes, W.K. and Dyer, R.S. (1983) Pattern reversal and flash evoked potentials following acute triethyltin exposure. Neurobehav. Toxicol. Teratol., 5:571-577

Boyes, W.K., Jenkins, D.E. and Dyer, R.S. (1985) Chlordimeform produces contrast dependent changes in visual evoked potentials of hooded rats. Exper. Neur. 89:391-407

Brenner, R.P. and Snyder, R.D. (1980) Late EEG findings and clinical status after organic mercury poisoning. Arch. Neurol. 37:282-284

Brown, A.W., Aldridge, W.N., Street, B.W. and Verschoyl, P.D. (1979) The behavioral and neuropathological sequelae of intoxication by trimethyltin compounds in the rat. Am. J. pathol., 97:59-82

Brown, A.W., Verschoyle, R.D., Street, B.W. and Aldridge, W.N. (1984) The neurotoxicity of trimethyltin chloride in hamsters, gerbils and marmosets. J. appl. Toxicol., 4:12-21

Browning, R.A. (1985) Role of the brain-stem reticular formation in tonic-clonic seizures: lesion and pharmacological studies. Fed. Proc. 44:2425-2431

Browning, R.A. and Nelson, D.K. (1984) Variation in threshold and pattern of electroshock-induced seizures in rats depending on site of stimulation. Neurosci. Abs. 10: 187

Byrd, J.T. and Andreae, M.O. (1982) Tin and methyltin species in seawater: Concentrations and fluxes. Science, 218: 565-569

Chang, L.W. and Dyer, R.S. (1983) Trimethyltin-induced pathology in sensory neurons. Neurobehav. Toxicol. Teratol., 5: 673-696

Chang, L.W. and Dyer, R.S. (1985) Septo-temporal gradients of trimethyltin- induced hippocampal damage. Neurobehav. Toxicol. Teratol., 7: 43-49

Chang, L.W. and Dyer, R.S. (1985) Early effects of trimethyltin on the dentate gyrus basket cells: a morphological study. J. Toxicol. Environ. Hlth. X: XXX-XXX

Chang, L.W., Wenger, G.R., McMillan, D.E., and Dyer, R.S. (1983) Species and strain comparison of acute neurotoxic effects of trimethyltin in mice and rats. Neurobehav. Toxicol. Teratol. 5: 337-350

Cinca, I., Dumitrescu, I., Onaca, P., Serbanescu, A. and Nestorescu, B. (1979) Accidental ethyl mercury poisoning with nervous system, skeletal muscle, and myocardium injury. J. Neurol. Neurosurg. Psychiat. 43: 143-149

Costa, L.G. (1985) Inhibition of gamma-[^{3}H]aminobutyric acid uptake by organotin compounds in vitro. Toxicol. appl. Pharmacol., 79: 471-479

Doctor, S.V., Costa, L.G., and Murphy, S.D.(1982) Effect of trimethyltin on chemically-induced seizures. Toxicol. Let. 13: 217-223

Doctor, S.V. and Fox, D.A. (1982) On the role of carbonic anhydrase in the anticonvulsive effects of triethyltin (TET). Experientia, 38, 824-826

Doctor, S.V. and Fox, D.A. (1982) Effects of organotin compounds on maximal electroshock seizure (MES) responsiveness in mice. I. Tri(n-alkyl)tin compounds. J. Toxicol. Environ. Health, 10: 43-52

Doctor, S.V. and Fox, D.A. (1982) Effects of organotin compounds on maximal electroshock seizure (MES) responsiveness in mice. II. tricyclohexyltin and triphenyltin. J. Toxicol. Environ. Health, 10: 53-58

Doctor, S.V. and Fox, D.A. (1983) Immediate and long-term alterations in maximal electroshock seizure responsiveness in rats neonatally exposed to triethyltin bromide. Toxicol. appl. Pharmacol., 68, 268-281

Dyer, R.S. (1985) The use of sensory evoked potentials in toxicology. Fund. appl. Toxicol., 5:24-40

Dyer, R.S. (1986a) Macrophysiological methods in neurobehavioral toxicology: the interface between behavior and neurophysiology in Z. Annau (Ed.) Neurobehavioral Toxicology, Baltimore, Johns Hopkins (in press)

Dyer, R.S. (1986b) Somatosensory evoked potentials. in H.H. Lowndes (Ed.) Electrophysiological endpoints in toxicology - neurotoxicology. CRC Press, in press

Dyer, R.S. and Boyes, W.K. (1983) Hypothermia and anesthesia differentially affect the flash evoked potentials of hooded rats . Brain Res. Bull. 10, 825-831

Dyer, R.S. and Boyes, W.K. (1984) Trimethyltin reduces recurrent inhibition. Neurobehav. Toxicol. Teratol. 6: 369-371

Dyer, R.S., Burden, E., Hulebak, K., Schultz, N., Swartzwelder, H.S. and Annau, Z. (1979) Hippocampal afterdischarges and their postictal sequelae in rats: a potential tool for assessment of CNS neurotoxicity. Neurobehav. Toxicol., 1, 5-19

Dyer, R.S., Eccles, C.U. and Annau, A. (1978) Evoked potential alterations following prenatal methyl mercury exposure. Pharmacol. Biochem. Behav., 8: 137-171

Dyer, R.S. and Howell, W.E. (1982a) Acute triethyltin exposure: effects on the visual evoked potential and hippocampal afterdischarge. Neurobehav. Toxicol. Teratol., 4: 259-266

Dyer, R.S. and Howell, W.E. (1982b) Triethyltin: ambient temperature alters visual system toxicity. Neurobehav. Toxicol. Teratol., 4: 267-271

Dyer, R.S., Howell, W.E., and Wonderlin, W.F. (1982a) Visual system dysfunction following acute trimethyltin exposure in rats. Neurobehav. Toxicol. Teratol., 4: 191-195

Dyer, R.S., Howell, W.E. and Reiter, L.W. (1981) Neonatal triethyltin exposure alters adult electrophysiology in rats. Neurotoxicol., 2: 609-623

Dyer, R.S., Walsh, J.T., Wonderlin, W.F. and Bercegeay, M. (1982b) The trimethyltin syndrome in rats. Neurobehav. Toxicol. Teratol., 4: 127-133

Dyer, R.S., Wonderlin, W.F. and Walsh, T .J. (1982c) Increased seizure susceptibility following trimethyltin intoxication in rats. Neurobehav. Toxicol. Teratol., 4: 203-208

Eccles, C.U. and Annau, Z. (1982) Prenatal methyl mercury exposure: II. alterations in learning and psychotropic drug sensitivity in adult offspring. Neurobehav. Toxicol. Teratol. 4: 377-382

Eccles, C.U., Rauen, K. and McCarren, M. (1981) Hippocampal afterdischarge characteristics in adult rats prenatally exposed to methylmercury. Soc. Neurosci. Abs. 7:894

Evans, B.N., Yonovitz, A., and Fox, D.A. (198x) Triethyltin-induced hearing loss: a study using pure tone auditory brainstem responses. Toxicologist, x: 169 (abs)

Fortemps, E., Amand, G., Bomboir, A., Lauwerys, R. and Laterre, E.C. (1978) Trimethyltin poisoning. Report of two cases. Int. Arch. Occup. Environ. Hlth. 41: 1-6

Fox, D.A. (1982) Pharmacological and biochemical evaluation of triethyltin's anticonvulsant effects. Neurobehav. Toxicol. Teratol., 4: 273-278

Fox, D.A. and Doctor, S.V. (1983) Triethyltin decreases maximal electroshock seizure severity in adult rats. Toxicol. appl. Pharmacol., 68:260-267

Friberg, L. (1971) Methyl mercury in fish. A toxicologic-epidemiologic evaluation of risks. Nord. Hyg. Tidskr. 1-364.

Gerren, R.A., Groswald, D.E. and Luttges, M.W. Triethyltin toxicity as a model for degenerative disorders. Pharmacol. Biochem. Behav., 5: 299-307, 1976.

Gloor, P., Ball, G. and Schaul, N. (1977) Brain lesions that produce delta waves in the EEG. Neurology, 27, 326-333.

Gordon, C.J., Long, M.D. and Dyer, R.S. Effect of triethyltin on autonomic and behavioral thermoregulation of mice. Toxicol. appl. Pharmacol., 73:543-550, 1984.

Graham, D.I., de Jesus, D.V., Pleasure, D.E. and Gonatas, N.K. Triethyltin sulfate-induced neuropathy in rats. Arch. Neurol., 33: 40-48, 1976.

Hallas, L.E., Means, J.C. and Cooney, J.J. (1982) Methylation of tin by estuarine microorganisms. Science, 215: 1505-1507.

Hasan, Z., Zimmer, L. and Woolley, D. (1984) Time course of the effects of trimethyltin on limbic evoked potentials and distribution of tin in blood and brain in the rat. Neurotoxicol. 5: 217-244.

Hetzler, B.E. and Dyer, R.S. Contribution of hypothermia to effects of chloral hydrate on flash evoked potentials of hooded rats. Pharmacol. Biochem. Behav., 21:599-607 1984.

Howell, W.F., Dyer, R.S., Wonderlin, W.F., Kidd, K.and Reiter, L.W. (1981) Sensory system effects of acute trimethyltin (TMT) exposure. Toxicologist, 1:43.

Howell, W.F., Walsh, T.J. and Dyer, R.S. (1982) Trimethyltin produces somatosensory dysfunction. Neurobehav. Toxicol. Teratol., 4:196-202.

Janssen, R.J. and Dyer, R.S. (1984) Effects of trimethyltin on auditory function in rats. Toxicologist 4: 119.

Janssen, R. Benignus, V., Grimes, L. and Dyer, R.S. (1976) Unrecognized errors due to analog filtering of the brainstem auditory evoked response. Electroencephalog. clin. Neurophysiol. (submitted)

Konishi, T. and Hamrick, P.E. (1979) The uptake of methyl mercury in guinea pig cochlea in relation to its ototoxic effect. Acta Otolaryngol. 88: 203-210

Loullis, C.C., Dean, R.L., Lippa, A.S., Clody, D.E., and Coupet, J. (1985) Hippocampal muscarinic receptor loss following trimethyl tin administration. Pharmacol. Biochem. Behav., 22: 147-151

Mattsson, J.L., Miller, E., Alligood, J.P., Koering, J.E. and Levin, S.G. (1981) Early effects of methylmercury on the visual evoked response of the dog. Neurotoxicol. 2: 499-514

Mazaev, V.T., Losev, N.I. and Voinov, V.A. (1968) Changes in the electroencephalogram and some autonomic reactions after poisoning with organotin compounds. Bull. exp. Biol. Med. 66: 72-74

Menashi, M., Ornoy, A., and Yanai, J. Transplacental effects of methylmercury chloride in mice with specific emphasis on the audiogenic seizure response. Dev. Neurosci. 5: 216-221

Merigan, W.H., Maurissen, J.P.J., Weiss, B., Eskin, T. and Lapham, L.W. (1983) Neurotoxic actions of methylmercury on the primate visual system. Neurobehav. Toxicol. Teratol., 5: 649-658

Miyami, T., Minowa, K., Seki, H. Tamura, Y. Mizoguchi, I. Ohi, G., and Suzuki, T. (1983) Chronological relationship between neurological signs and electrophysiological changes in rats with methylmercury poisoning - special reference to selenium protection. Arch. Toxicol., 52:173-181

Mukuno K., Ishikawa S. and Okamura, R. (1981) Grating test of contrast sensitivity in patients with Minimata disease. Brit. J. Ophthalmol. 65: 284-290.

Murai, Y., Shiraishi S., Yamashita Y.U., Ohnishi, A., and Arimura, K. (1982) Neurophysiological effects of methyl mercury on the nervous system. Electroencephalog. clin. Neurophysiol. Suppl. 36: 682-687.

Nosaka Y. Sadanaga, M., Shiga A., Ohgi H., Asano, S., Kiyofuji, T. and Togashi,N. (1970) Auditory, vestibular, gustatory and speech disturbances in Minimata disease Jap. J. Otol. (Tokyo) 73: 1006.

Pinel, J.P.J , Treit, D., and Rovner, L.I. (1977) Temporal lobe aggression in rats. Science. 197: 1088-1089.

Pruill, G. and Rompel, K. (1970) EEG changes in acute poisoning with organic tin compounds. Electroencephalog. clin. Neurophysiol., 29:215

Ray, D.E. (1981) Electroencephalographic and evoked response correlates of trimethyltin induced neuronal damage in the rat hippocampus. J. appl. Toxicol., 1: 145-148.

Reiter, L.W., Heavner G.B. Dean, K.F., and Ruppert, P.H. (1981) Developmental and behavioral effects of early postnatal exposure to triethyltin in rats Neurobehav. Toxicol. Teratol., 3 285-293

Ross, W.D.., Emmett, E.A., Steiner, J. and Tureen, R. (1981) Neurotoxic effects of occupoational exposure to organotins. Am. J. Psychiat. 138:1092-1095

Ruppert, P.H., Dean, K.F and Reiter. L.W. (1984) Trimethyltin disrupts acoustic startle responding in adult rats. Toxicol. Let. 22:33-38

Saito, K. (1973) Electroencephalographic studies on petrol intoxication: comparison between nonleaded and leaded white petrol. Brit. J. Indust. Med. 30: 352-358.

Scheinberg, L.C., Taylor, J.M., Herzog, I. and Mandell, S. (1966) Optic and peripheral nerve response to triethyltin intoxication in the rabbit: biochemical and ultrastructural studies. J. Neuropath. exp. Neurol. 25: 202-213

Selwyn, W.J. (1976) Triorganotin compounds as ionophores and inhibitors of ion translocating ATPases. in J.J. Zuckerman (Ed.) Organotin compounds: New Chemistry and applications. American Chemical Society, Washington, D.C. 204-226.

Selwyn, M.J., Dawson, A.P., Stockdale, M. and Gains, N. (1970) Chloride-hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes mediated by trialkyl- and triphenyltin compounds, Eur. J. Biochem. 14: 120-126

Shah, S.N., Johnson, R.C., Amochaev, A. and Salamy, A. (1978) Effects of triethyltin induced neuropathy on brain stem auditory evoked potentials in rats. Neuroscience Abs. 4: 156 (abs)

Shelton, W.W., Nevis, A.H. and Collins, G.H. (1982) Triethyltin toxicity and brain impedence. Acta Neuropathol. 58: 95-100.

Sloviter, R.S. and Damiano, B.P. (1981a) Sustained electrical stimulation of the perforant path duplicates kainate-induced electrophysiological effects and hippocampal damage in rats. Neurosci. Lett. 24: 279-284.

Sloviter, R.S. and Damiano, B.P. (1981b) On the relationship between kainic acid-induced epileptiform activity and hippocampal neuronal damage. Neuropharmacol. 20: 1003-1011.

Sloviter, R.S., Becker, C., Walsh, T.J., and Dempster, D.W. (1985) The role of seizure activity in the hippocampal damage produced by trimethyltin. Brain Research XXX: XXX-XXX

Su, M-Q and Okita, G.T. (1976) Behavioral effects on the progeny of mice treated with methylmercury. Toxicol. appl. Pharmacol. 38: 195-205

Torack, R., Gordon, J. and Prokop, J. (1970) Pathobiology of acute triethyltin intoxication. Int. Rev. Neurobiol., 12, 45-86

Traub, R.D., Dudek, F.E. Taylor, C.P. and Knowles, W.D. (1985) Simulation of hippocampal afterdischarges synchronized by electrical interactions. Neurosci. 14: 1033-1038

Von Burg, R. and Rustam, H. (1974) Electrophysiological investigations of methylmercury intoxication in humans. Evaluation of peripheral nerve by conduction velocity and electromyography. Electroencephalog. clin. Neurophysiol 37:381-392

Wassick, K.H. and Yonovitz, A. (1985) Methyl mercury ototoxicity in mice determined by auditory brainstem responses. Acta Otolaryngol. 99: 35-45

Wenger, G.R., McMillan D.E., and Chang, L.W. (1984) Behavioral effects of trimethyltin in two strains of mice I. spontaneous motor activity. Toxicol. appl. Pharmacol., 73: 78-88

Whishaw, I.Q. and Vanderwolf, C.H. (1973) Hippocampal EEG and behavior: Changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behav. Biol. 8:461-484

Woodbury, L.A. and Davenport, V.D. (1952) Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. Arch. int. Pharmacodyn. 92: 97-107

Wu, M-F., Ison, J.R. Wecker, J.R. and Lapham, L.W. (1985) Cutaneous and auditory function in rats following methyl mercury poisoning. Toxicol. appl. Pharmacol. 79: 377-388.

Zenick, H. (1974) Behavioral and biochemical consequences in methylmercury chloride toxicity. Pharmacol. Biochem. Behav. 2: 709-713.

Zenick, H. (1976) Evoked potential alterations in methylmercury chloride toxicity. Pharmacol. Biochem. Behav. 5: 253-255.

Zook, B.C. and Wilpizeski, C.R. (1980) Functional and morphological changes in the auditory system of nonhuman primates during chronic methylmercury poisoning. Toxicol. Let 6 (Special edition): 135.

Figure Legends

Figure 1. Effects of methylmercury on sleep in Sprague-Dawley rats. A. Effects of exposure on total sleep time. B. Effects of exposure on the distribution of waking (W) slow wave sleep (SWS) and paradoxical sleep (PS) Dotted lines show the values obtained by averaging across the 4 pre-administration days. Open and filled bars shown at the bottom indicate the time scale of a 12:12 hr light/dark cycle. These data are redrawn from Arito et al. (1983).

Figure 2. Effects of daily oral exposures to 0.5 mg/kg methylmercury on a derived measure of flash evoked potentials recorded from unanesthetized dogs. S^2 is the variance obtained from difference graphs of successive waveforms. r is the correlation between graphs and F is the ratio of [S^2 larger/ S^2 smaller] for the two days involved in the comparison. S^2 evaluates amplitudes, while $r/F_{1/2}$ accounts for shape and magnitude. Graph redrawn from Mattsson et al. (1981).

Figure 3. Effects of methylmercury on pure tone thresholds in rats, as determined with the brainstem auditory evoked response (BAER). The 4 mg/kg group received 17 daily doses (total dose 68 mg/kg, and the 8 mg/kg group received 6 daily doses (total dose 48 mg/kg). The data, replotted from Wassick and Yonovitz (1985), indicate an apparent loss of sensitivity at all frequencies.

Figure 4. Effects of methylmercury on BAER interpeak latencies (Peak IV-Peak I), an indicator of central rather than peripheral dysfunction. Data are plotted for the 16 KHz stimulus. The data suggest decreased central conduction time (increased speed). Data obtained from Wassick and Yonovitz (1985).

Figure 5. FEPs recorded from (A) one rat in the saline control group during the first 9 days of the experiment; and (B) one rat in the 1.5 mg TET/kg/day dosage group during the first 9 days of the experiment. Figure reprinted from Dyer and Howell (1982a).

Figure 6. Time course of changes in N1 latency of FEPs recorded from rats following administration of 1.5 mg/kg TET daily on days 2-7. N1 latency continued to increase following termination of dosing and did not recover until after day 25. Figure reprinted from Dyer and Howell (1982a).

Figure 7. Time and temperature-dependent nature of effects produced by a single injection of 6 mg/kg TET bromide upon the latency of FEP peak N1. Top: animals maintained at "cool" (22°C) room temperature during the period of temperature control. Differences between TET and control rats were absent 24 hrs following exposure. Bottom: animals maintained in a warm (30°C) environment during the period of temperature control. Differences between TET and control rats were still evident 300 hrs following exposure. Figure reprinted from Dyer and Howell (1982b).

Figure 8. Effects of TET on postictal EEG depression. Magnitude of post-AD depression was calculated using integrated EEG activity. The Figure, redrawn from Dyer and Howell (1982a), illustrates an increased postictal depression resulting from exposure to TET.

Figure 9. Effects of 10 daily exposures to TET in the drinking water on percentage of rats showing a grade 5 MES. The data illustrate a depressant/anti-convulsant effect of TET. The data were obtained from Fox and Doctor (1983).

Figure 10. Influence of postnatal day 5 exposures to TET on mean (±SEM) FEP latencies recorded from unanesthetized adult rats. * = different from saline control. Reprinted from Dyer et al. (1981).

Figure 11. Effects of early postnatal exposure to TET on development of stage 5 MESs in female rats. Rats were given the indicated dosage on postnatal days 0, 5, 10, 15 and 20. Therefore total exposures were 0, 1.25, 2.5, 5, 12.5 and 25 mg/kg. The data obtained from Doctor and Fox (1983) indicate a TET-induced developmental delay in appearance of stage 5 MESs, particularly if the normal rate is taken to be about 60%.

Figure 12. Effects of early postnatal exposure to TET on duration of hindlimbs extension (mean ± SEM) produced during MESs. Intermediate dosage groups appear to have more severe seizures. Data from Doctor and Fox (1983).

Figure 13. Motor conduction velocity (mean ± SEM) recorded following stimulation of the sciatic nerve in rats during (solid line) or after (dotted line) receiving 20 mg/L TET sulfate in their drinking water. Data obtained from Graham et al. (1976).

Figure 14. Power spectral analysis of the mouse EEG obtained at the indicated times following administration of 8 mg/kg TET. Solid lines are control values, dotted lines were obtained from TET-treated mice. Redrawn from Gerren et al. (1976).

Figure 15. FEP latencies recorded from mice at different times following administration of 8 mg/kg TET. The data, replotted from Gerren et al (1976), probably reflect in part the TET-induced hypothermia reported by others.

Figure 16. Effects of TMT upon FEPs recorded from the optic tract and visual cortex of rats. A. Amplitude of the two components of the initial wave of the FEP. B. latency of the onset of activity. Both sets of amplitude and latency data suggest retinal dysfunction. Data obtained from Dyer et al. (1982).

Figure 17. percentage change ± SEM (relative to pre-dose means) in sound-induced evoked potentials recorded from the rat hippocampus following administration of TMT. Solid line: TMT-treated. Dotted line: control. Data re-plotted from Ray (1981).

Figure 18. Percentage incidence of 3 characteristics of pentylenetetrazol seizures induced in rats 5 days following administration of TMT. Data from Dyer et al. (1982).

Figure 19. Development of kindling over blocks of 5 days after a single administration of TMT. Amygdaloid kindling is more rapid than hippocampal kindling, which was not fully developed even at the 30th day of stimulation. In both cases, TMT-treated rats kindled more rapidly than controls. Figure reprinted from Dyer et al. (1982).

Figure 20. Effects of TMT on recurrent inhibition in the dentate gyrus of rats. R1 is the amplitude of the population spike produced in the dentate gyrus by the first of paried stimuli presented to the perforant path. R2 is the amplitude of the population spike produced by the second of the paired stimuli. The smaller the ratio of R2/R1, the greater the recurrent inhibition. Values plotted are means ± SEM. Replotted from Dyer and Boyes (1984).

Figure 21. Amplitude of monosynaptic (DG-CA3) and polysynaptic (ppc-DG) evoked potentials recorded from an unanesthetized rat following administration of 7.5 mg/kg TMT. The polysynaptic pathway illustrates potentiation followed by depression, while the monosynaptic pathway shows only depression. Replotted from Hasan et al. (1984).

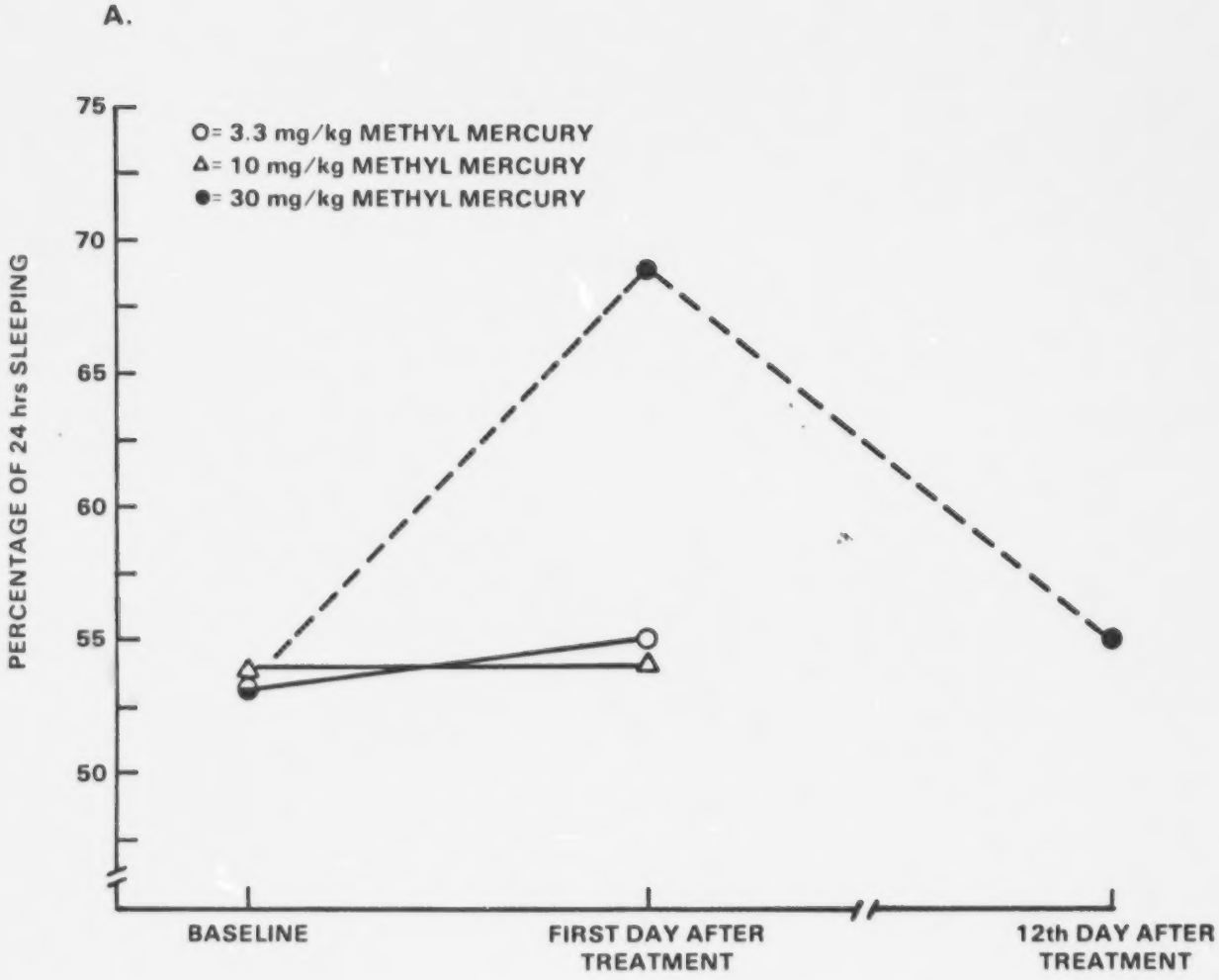

A.
O= 3.3 mg/kg METHYL MERCURY
Δ= 10 mg/kg METHYL MERCURY
●= 30 mg/kg METHYL MERCURY
PERCENTAGE OF 24 hrs SLEEPING
75
70
65
60
55
50
BASELINE
FIRST DAY AFTER TREATMENT
12th DAY AFTER TREATMENT

Fig 1 A

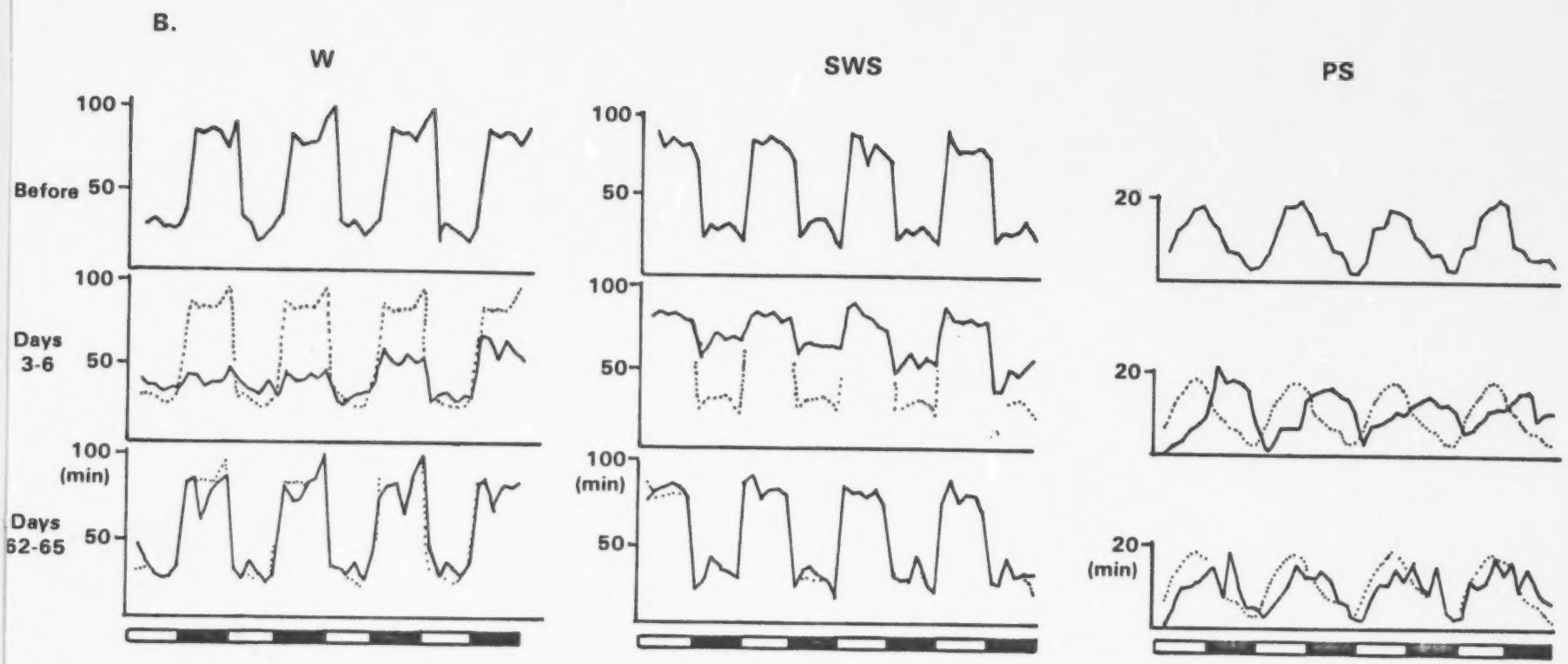


Fig 1 B

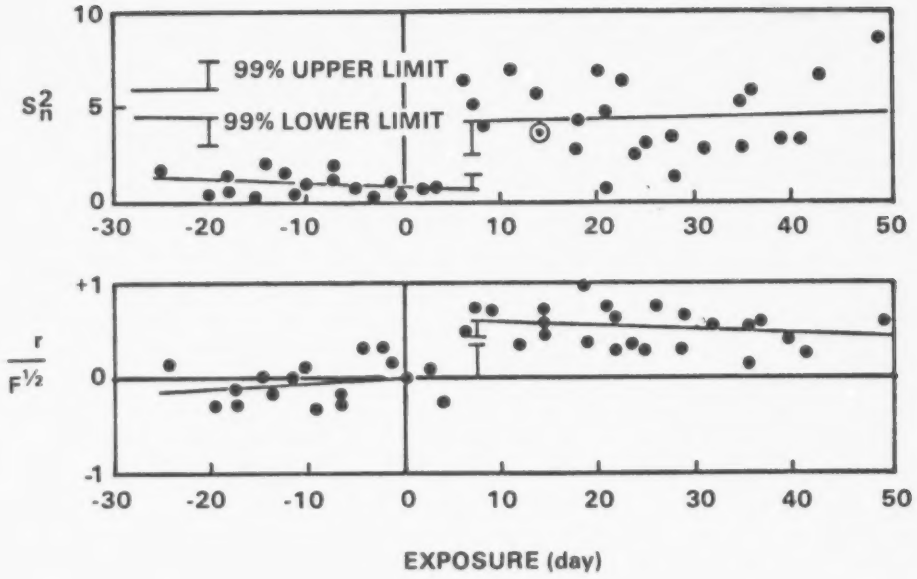

10
5
0
S_n^2
99% UPPER LIMIT
99% LOWER LIMIT
-30
-20
-10
0
10
20
30
40
50
+1
0
-1
$\frac{r}{F^{1/2}}$
EXPOSURE (day)

Fig 2

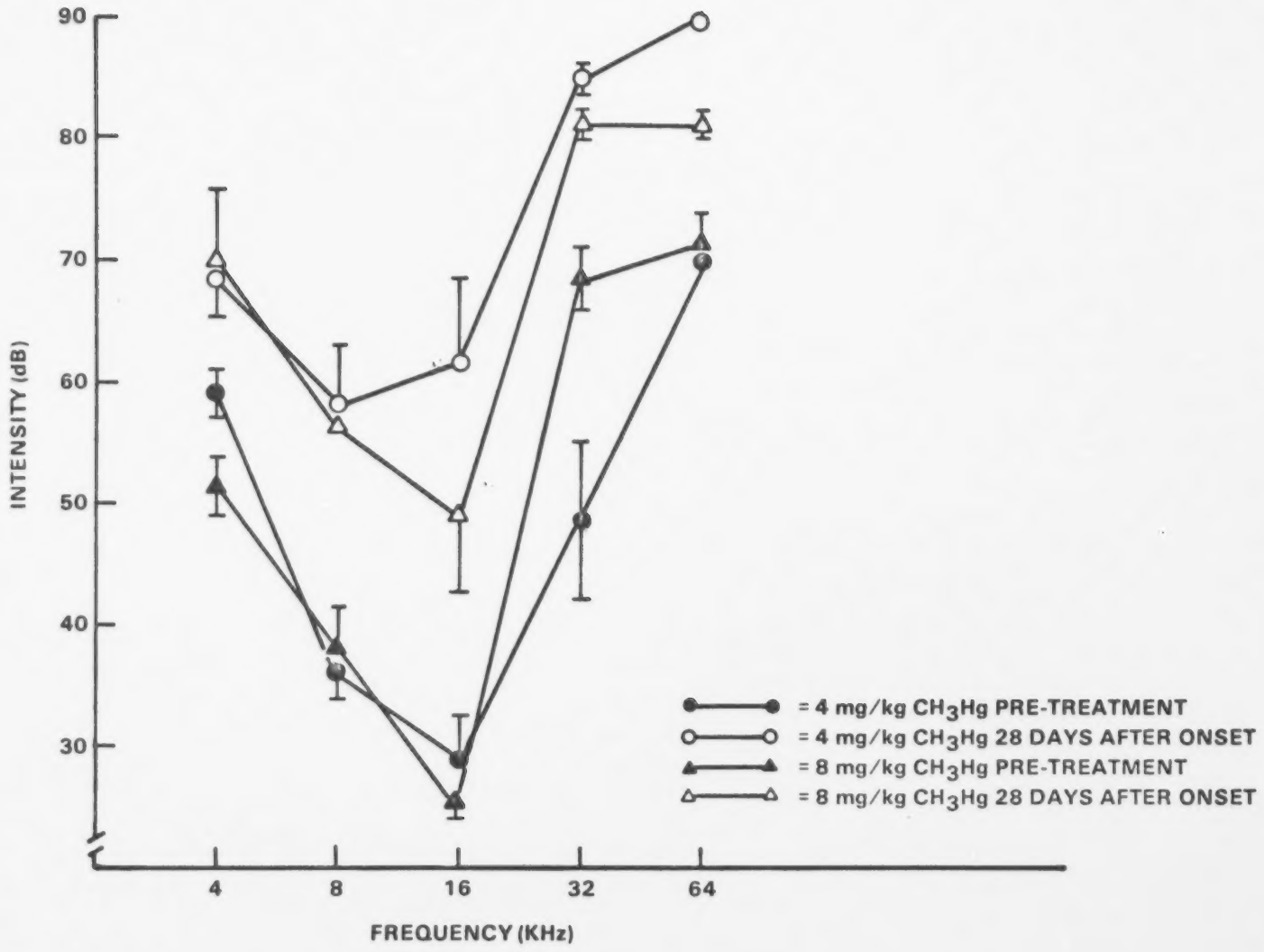

90
80
70
60
50
40
30
INTENSITY (dB)
4
8
16
32
64
FREQUENCY (KHz)
= 4 mg/kg CH3Hg PRE-TREATMENT
= 4 mg/kg CH3Hg 28 DAYS AFTER ONSET
= 8 mg/kg CH3Hg PRE-TREATMENT
= 8 mg/kg CH3Hg 28 DAYS AFTER ONSET

Fig 3

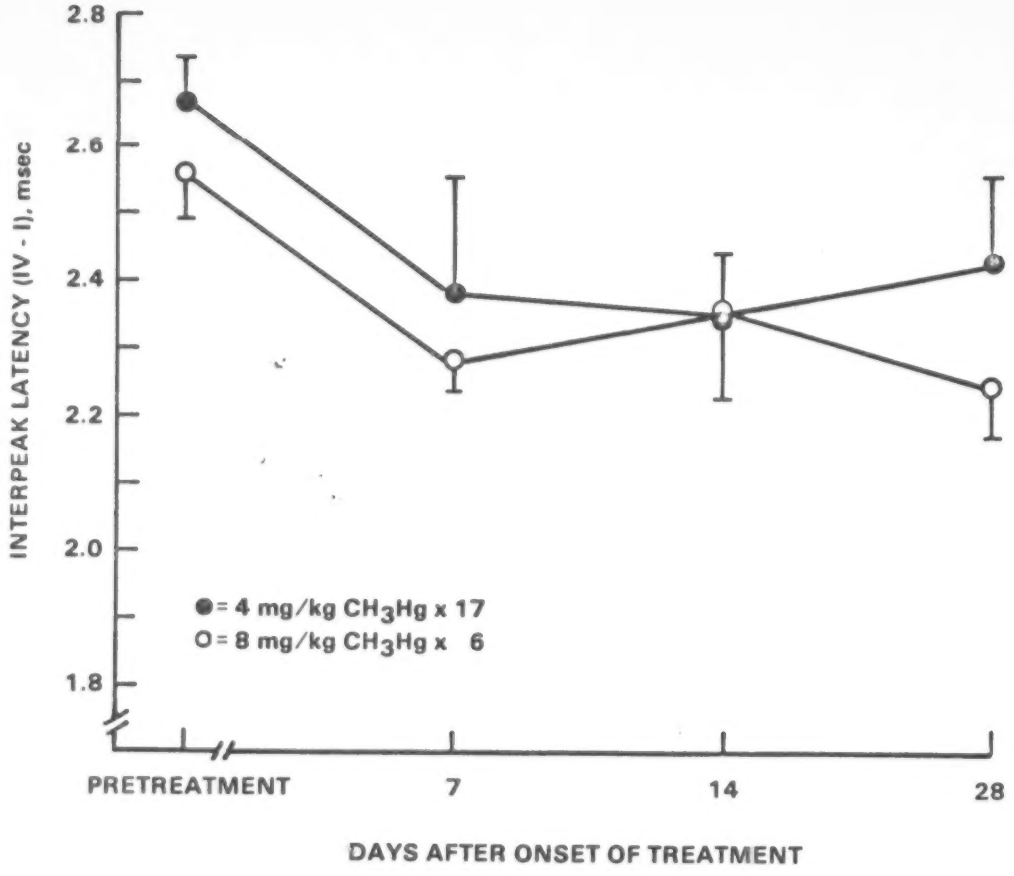

2.8
2.6
2.4
2.2
2.0
1.8
INTERPEAK LATENCY (IV - I), msec
● = 4 mg/kg CH_3Hg x 17
○ = 8 mg/kg CH_3Hg x 6
PRETREATMENT
7
14
28
DAYS AFTER ONSET OF TREATMENT

Fig 4

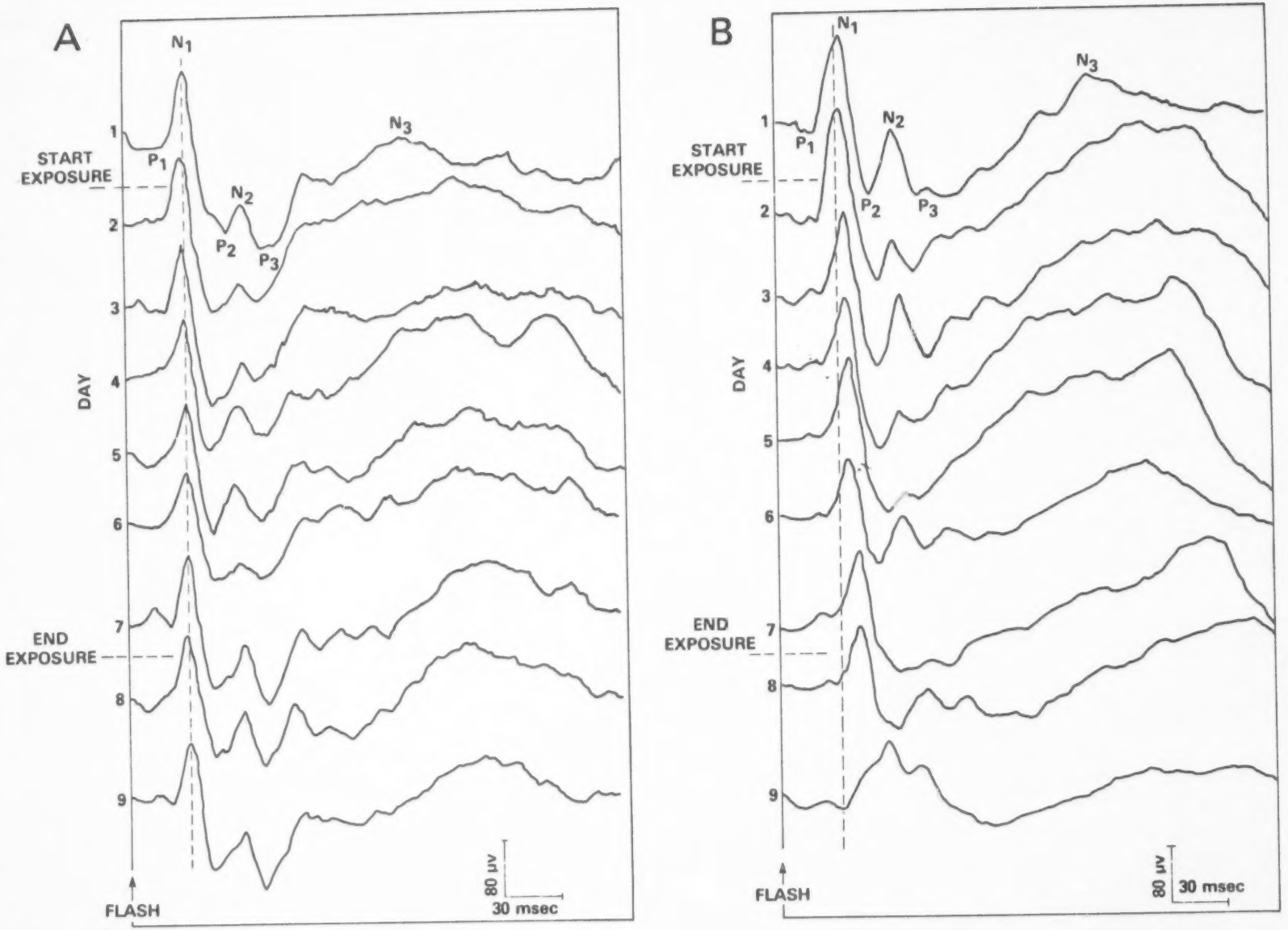

A
B
N_1
P_1
N_2
P_2
P_3
N_3
START EXPOSURE
END EXPOSURE
DAY
1
2
3
4
5
6
7
8
9
FLASH
80 µv
30 msec

FIG 5

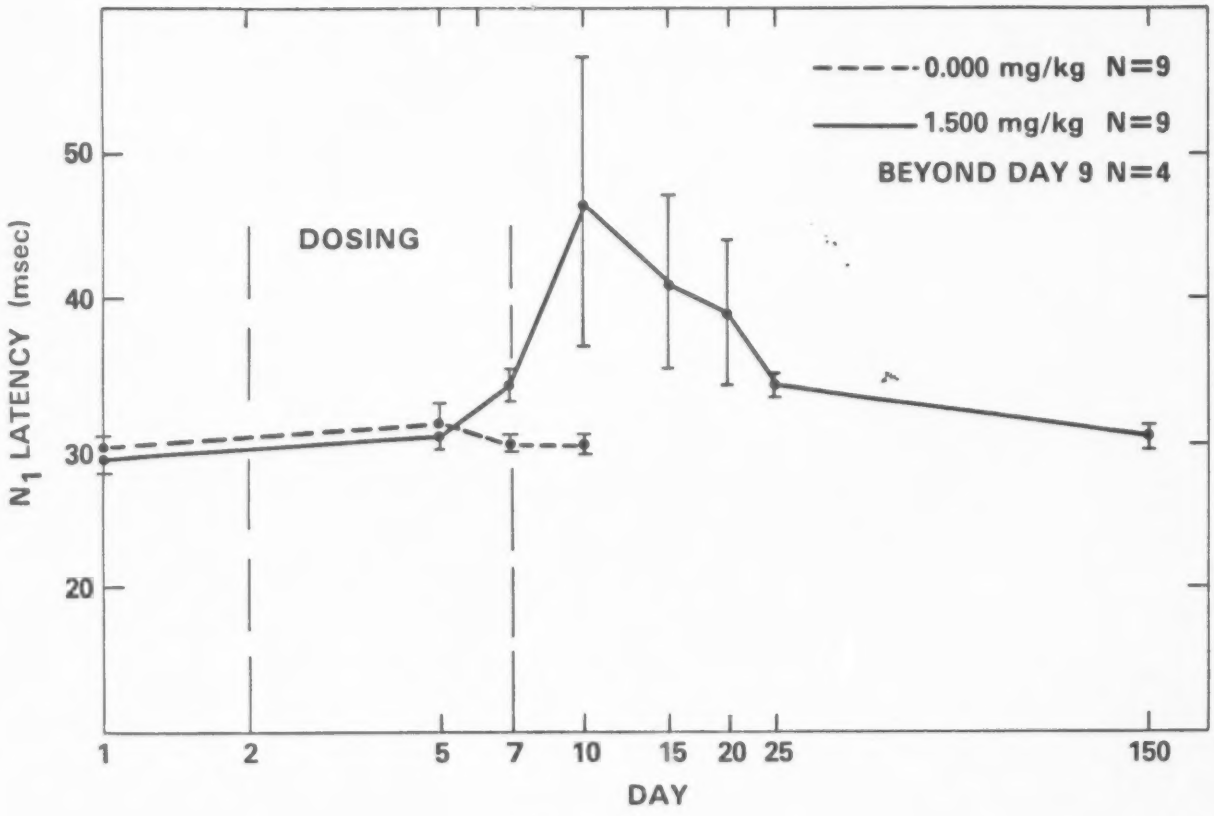

0.000 mg/kg N=9
1.500 mg/kg N=9
BEYOND DAY 9 N=4
DOSING
N_1 LATENCY (msec)
50
40
30
20
1
2
5
7
10
15
20
25
150
DAY

FIG 6

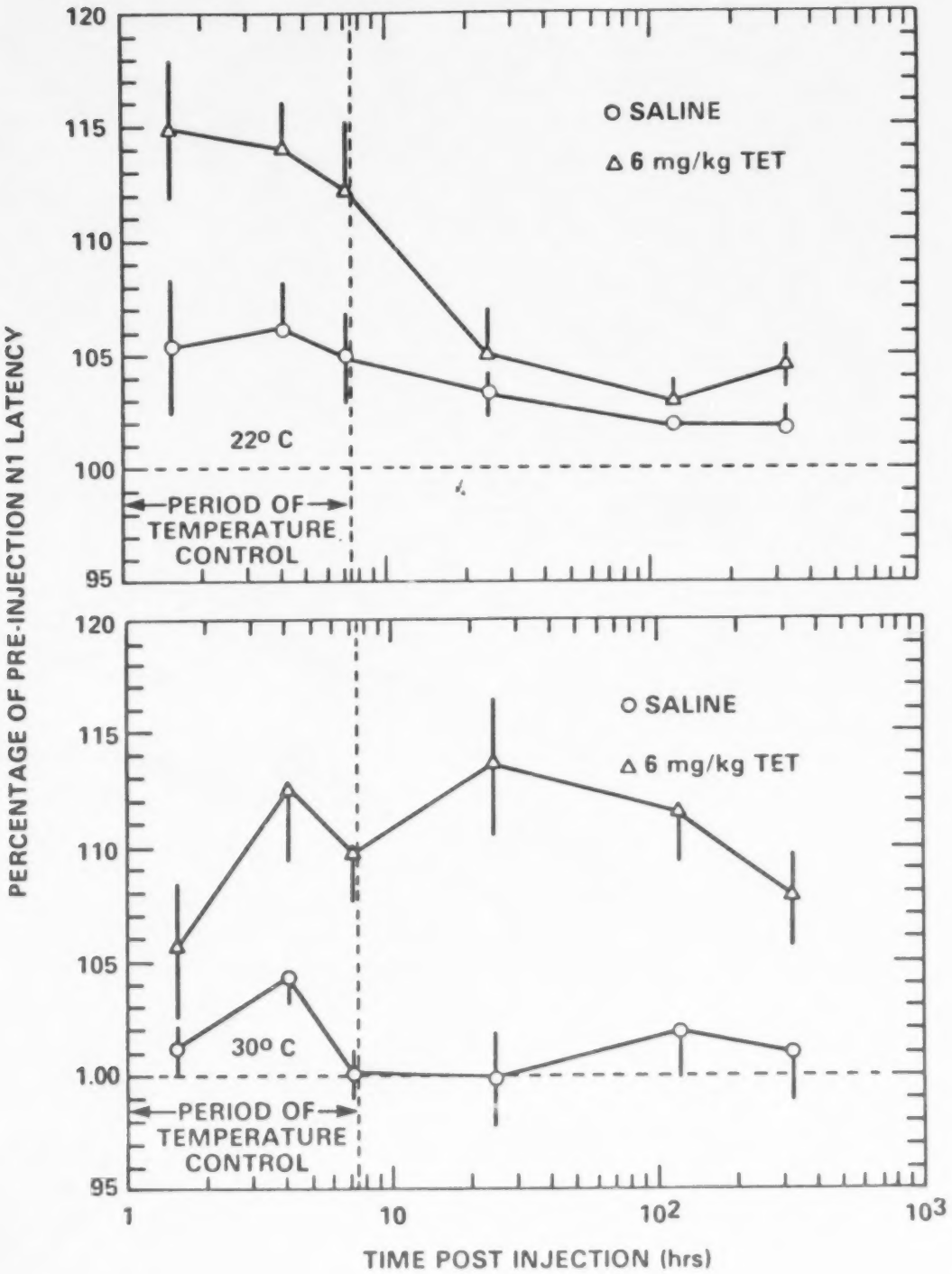

120
115
110
105
100
95
O SALINE
Δ 6 mg/kg TET
22° C
PERIOD OF TEMPERATURE CONTROL
120
115
110
105
1.00
95
O SALINE
Δ 6 mg/kg TET
30° C
PERIOD OF TEMPERATURE CONTROL
1
10
10²
10³
PERCENTAGE OF PRE-INJECTION N1 LATENCY
TIME POST INJECTION (hrs)

FIG 7

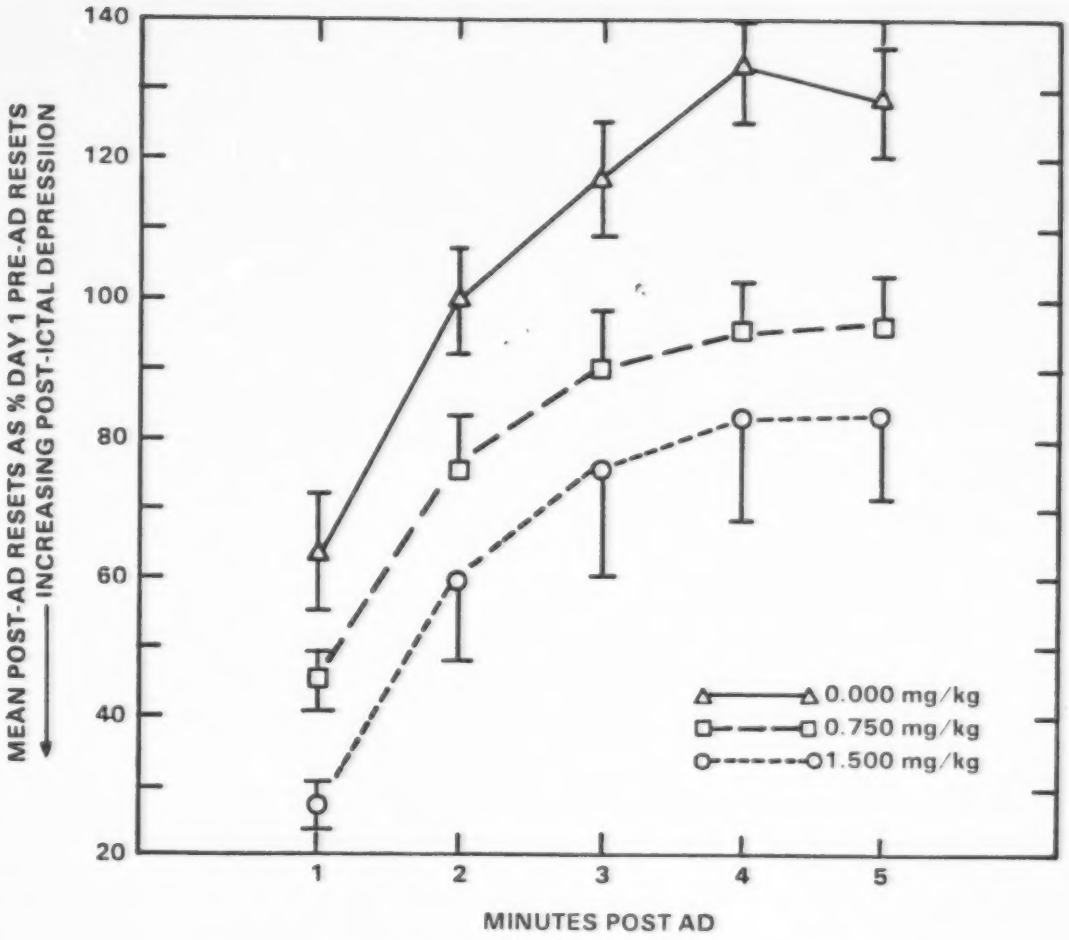

MEAN POST-AD RESETS AS % DAY 1 PRE-AD RESETS
INCREASING POST-ICTAL DEPRESSIION
140
120
100
80
60
40
20
1
2
3
4
5
MINUTES POST AD
0.000 mg/kg
0.750 mg/kg
1.500 mg/kg

Fig 8

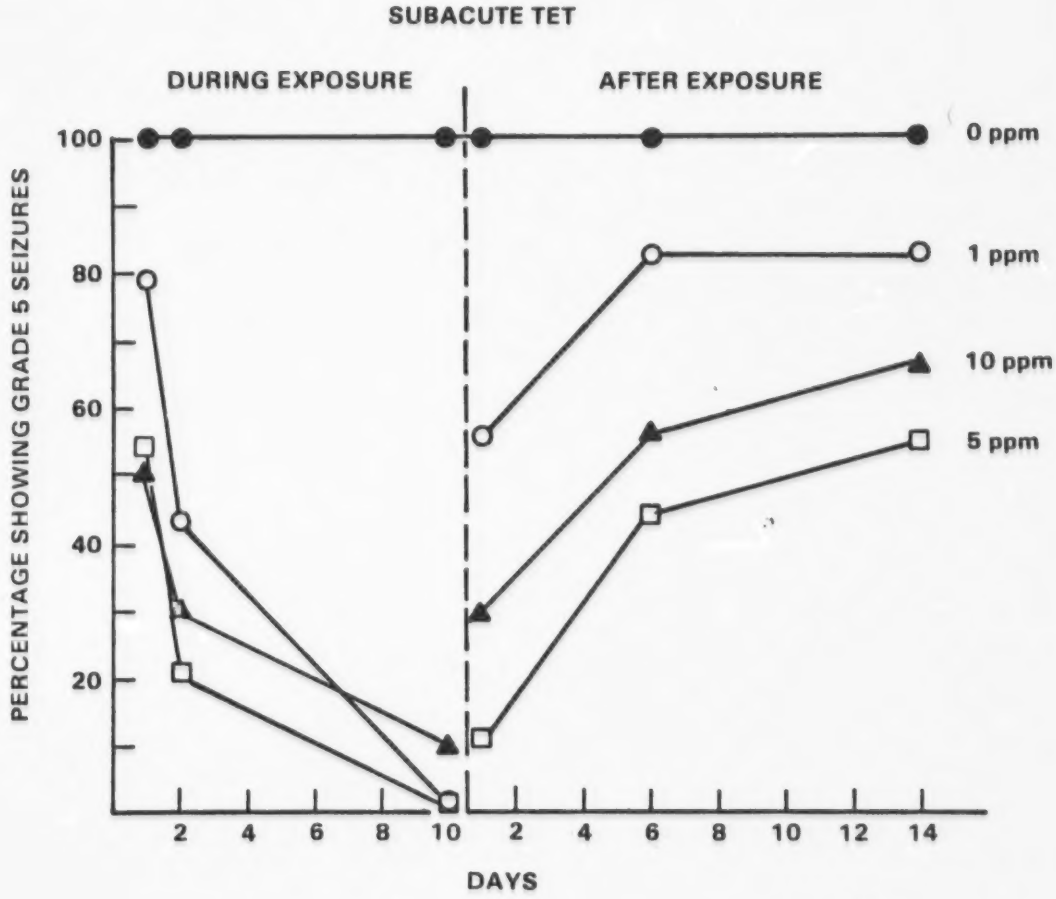

SUBACUTE TET
DURING EXPOSURE
AFTER EXPOSURE
PERCENTAGE SHOWING GRADE 5 SEIZURES
100
80
60
40
20
0 ppm
1 ppm
10 ppm
5 ppm
2
4
6
8
10
2
4
6
8
10
12
14
DAYS

Fig 9

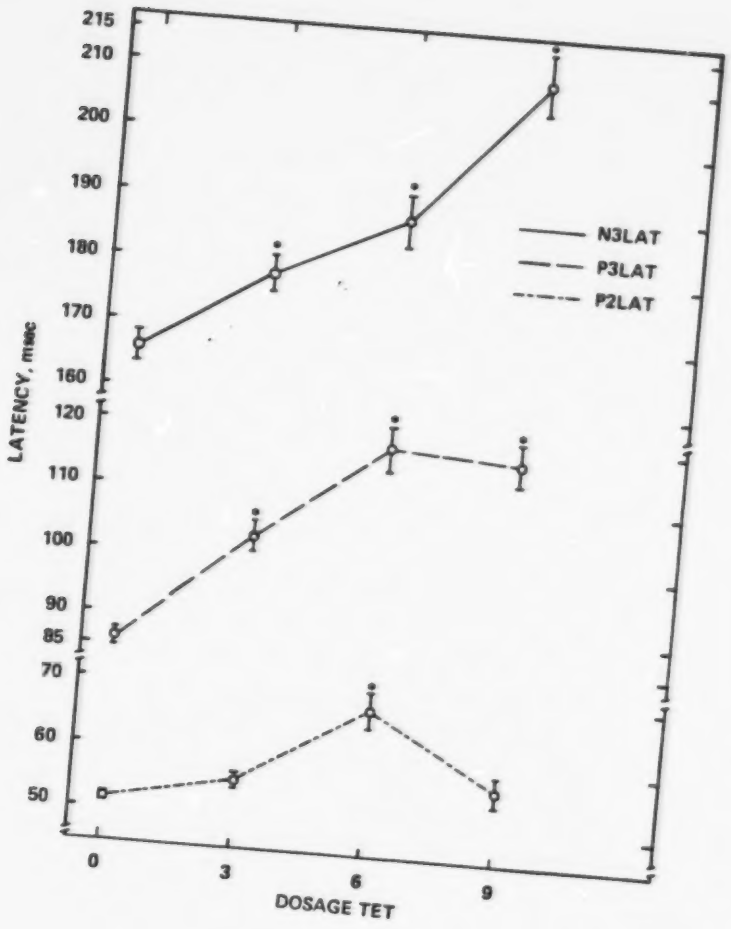

215
210
200
190
180
170
160
120
110
100
90
85
70
60
50
LATENCY, msec
N3LAT
P3LAT
P2LAT
0
3
6
9
DOSAGE TET

Fig 10

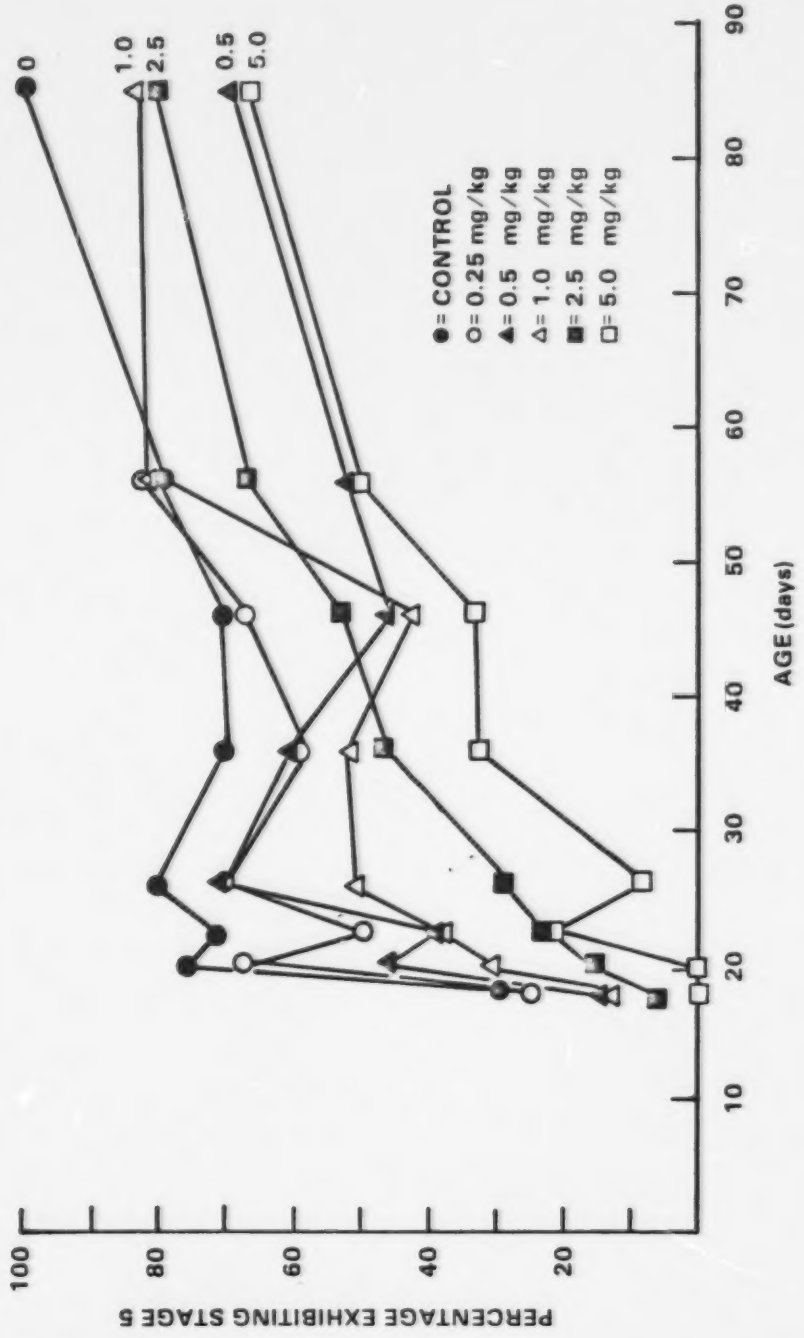

PERCENTAGE EXHIBITING STAGE 5
100
80
60
40
20
10
20
30
40
50
60
70
80
90
AGE (days)
● = CONTROL
○ = 0.25 mg/kg
▲ = 0.5 mg/kg
△ = 1.0 mg/kg
■ = 2.5 mg/kg
□ = 5.0 mg/kg
0
1.0
2.5
0.5
5.0

Fig 11

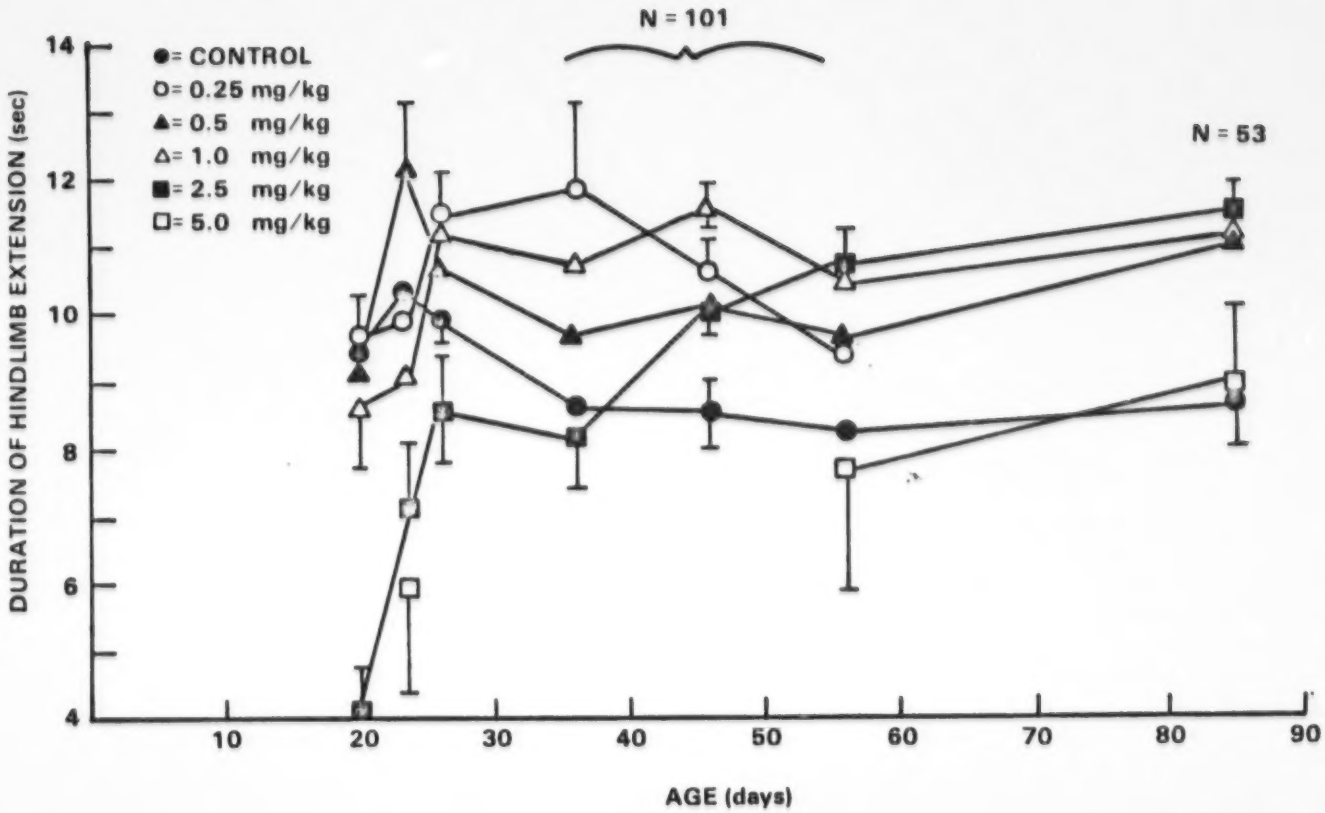

N = 101
N = 53
●= CONTROL
○= 0.25 mg/kg
▲= 0.5 mg/kg
△= 1.0 mg/kg
■= 2.5 mg/kg
□= 5.0 mg/kg
DURATION OF HINDLIMB EXTENSION (sec)
14
12
10
8
6
4
10
20
30
40
50
60
70
80
90
AGE (days)

FIG 12

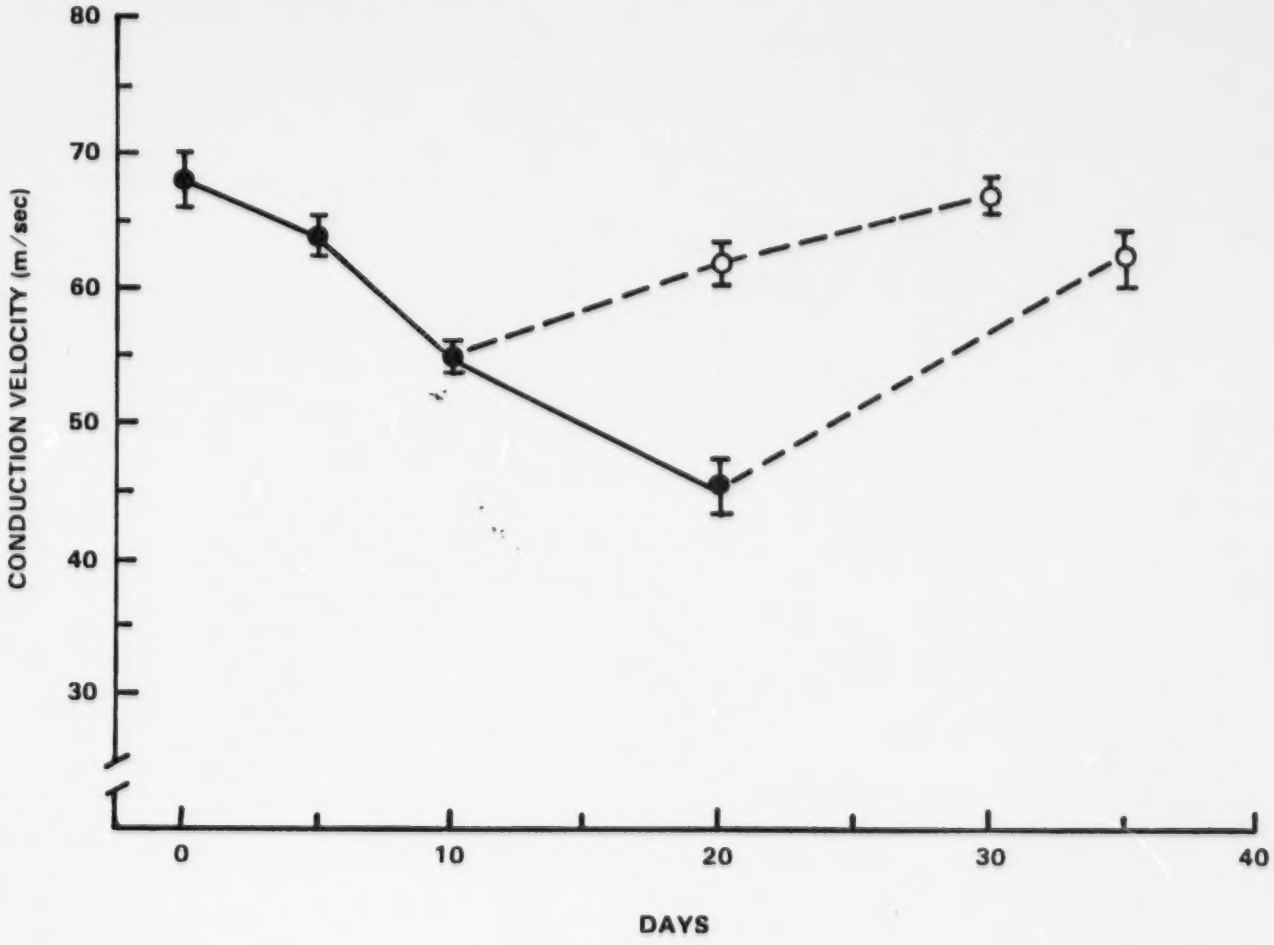


FIG 13

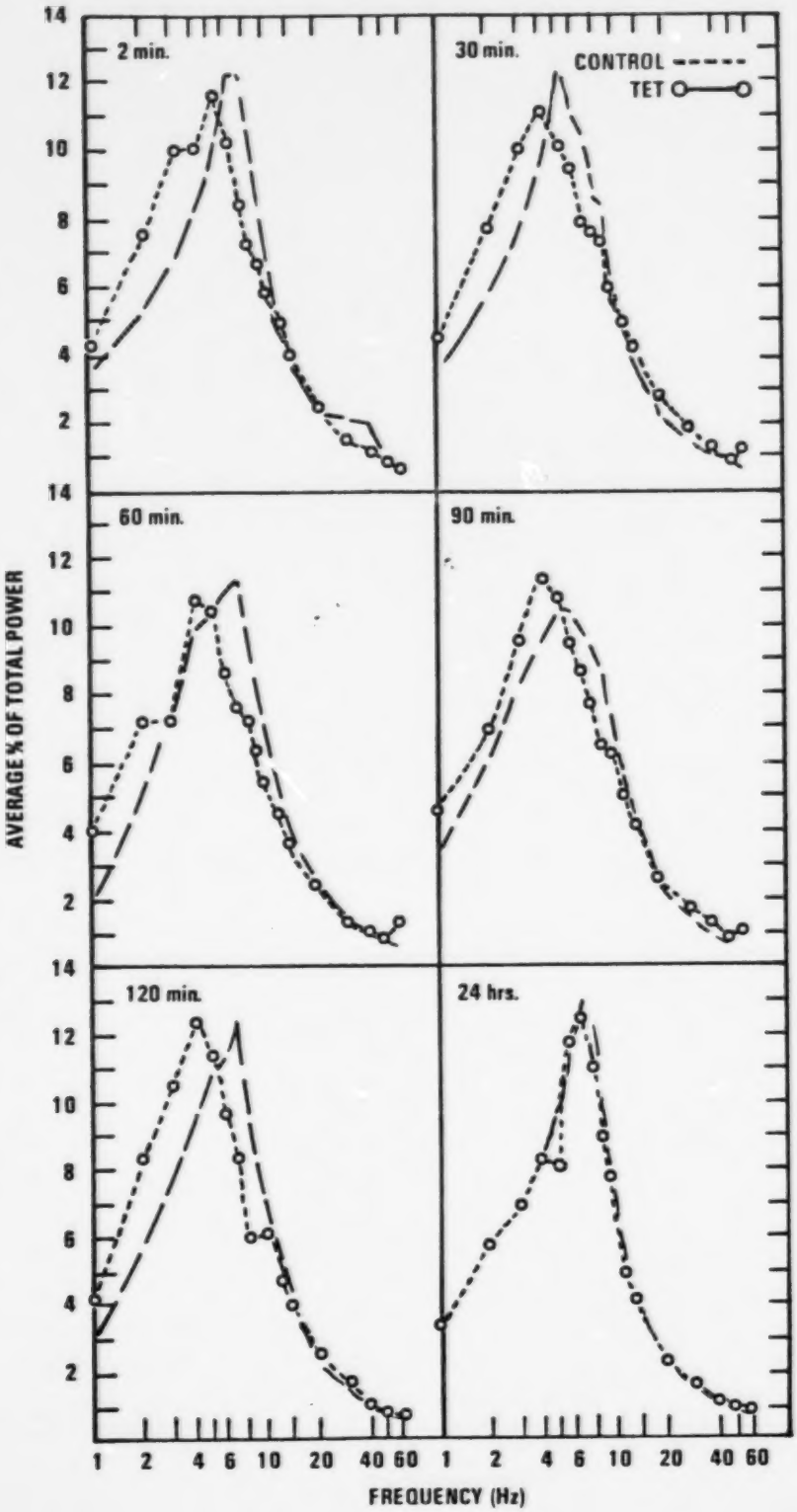

2 min.
30 min.
CONTROL
TET
60 min.
90 min.
120 min.
24 hrs.
AVERAGE % OF TOTAL POWER
FREQUENCY (Hz)
1 2 4 6 10 20 40 60
2 4 6 8 10 12 14

FIG 14

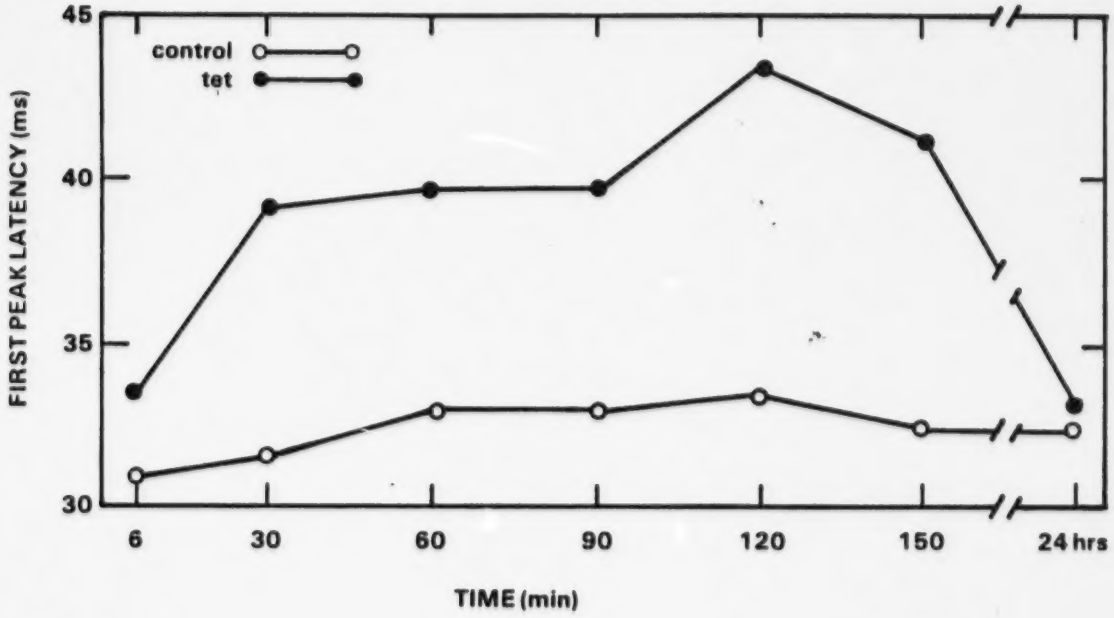

45
40
35
30
control
tet
FIRST PEAK LATENCY (ms)
6
30
60
90
120
150
24 hrs
TIME (min)

Fig 15

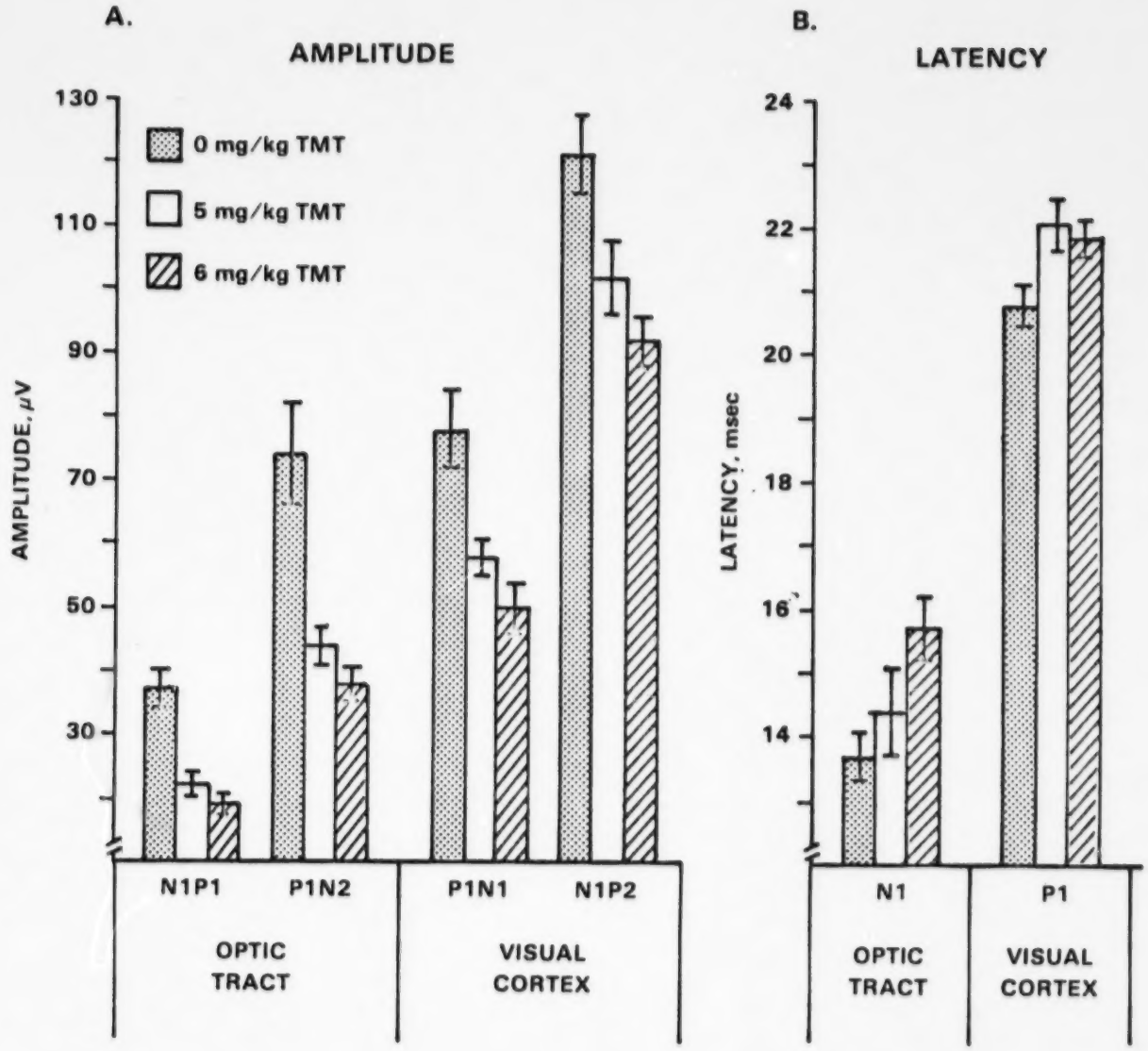

A.
AMPLITUDE
0 mg/kg TMT
5 mg/kg TMT
6 mg/kg TMT
AMPLITUDE, μV
130
110
90
70
50
30
N1P1
P1N2
P1N1
N1P2
OPTIC TRACT
VISUAL CORTEX
B.
LATENCY
LATENCY, msec
24
22
20
18
16
14
N1
P1
OPTIC TRACT
VISUAL CORTEX

FIG 16

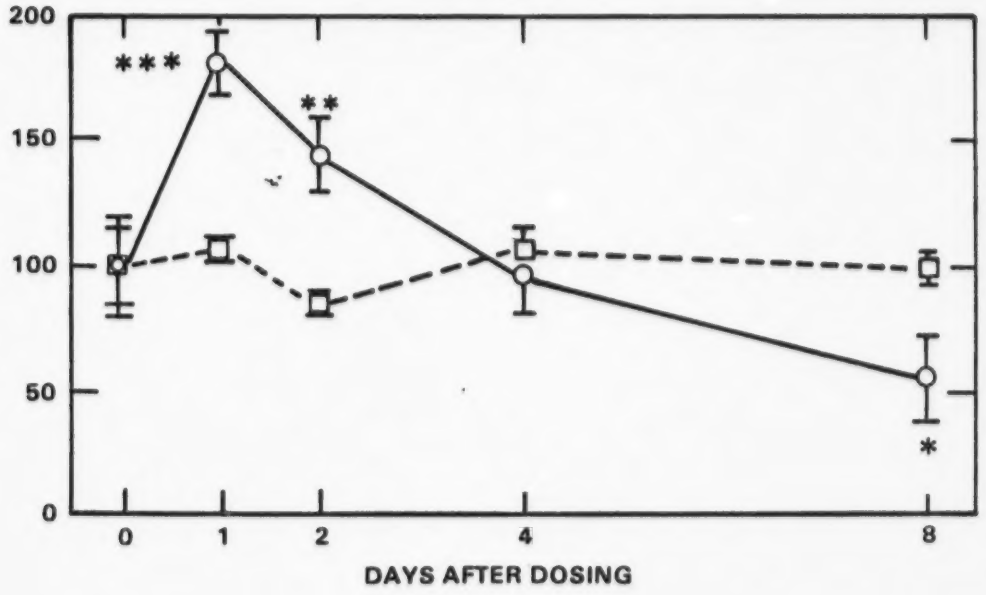


FIG 17

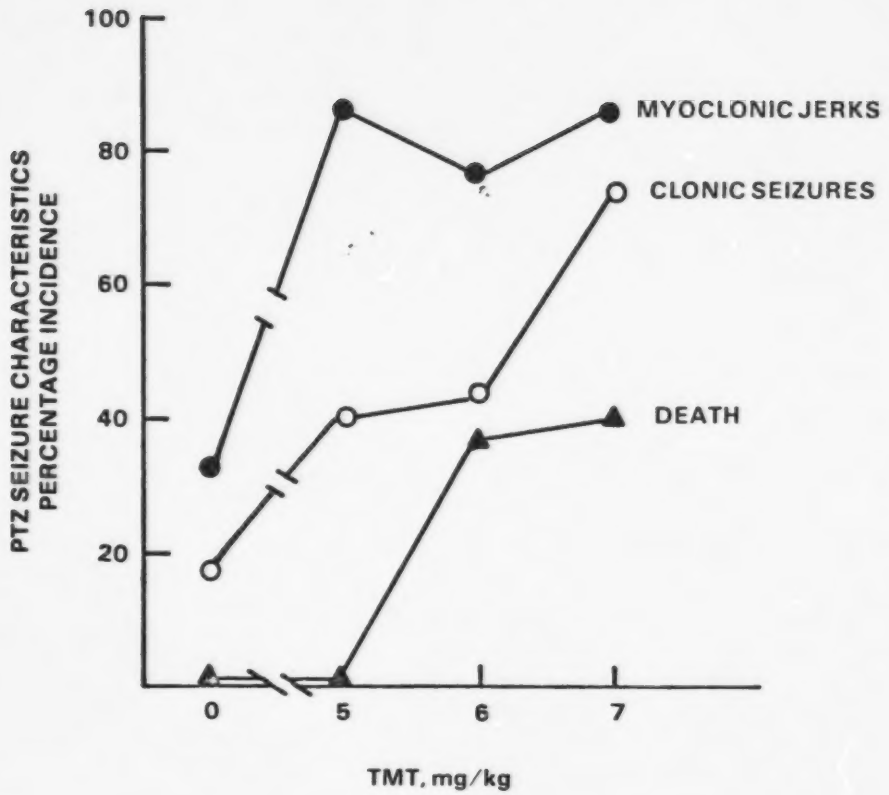

100
80
60
40
20
PTZ SEIZURE CHARACTERISTICS
PERCENTAGE INCIDENCE
MYOCLONIC JERKS
CLONIC SEIZURES
DEATH
0
5
6
7
TMT, mg/kg

FIG 18

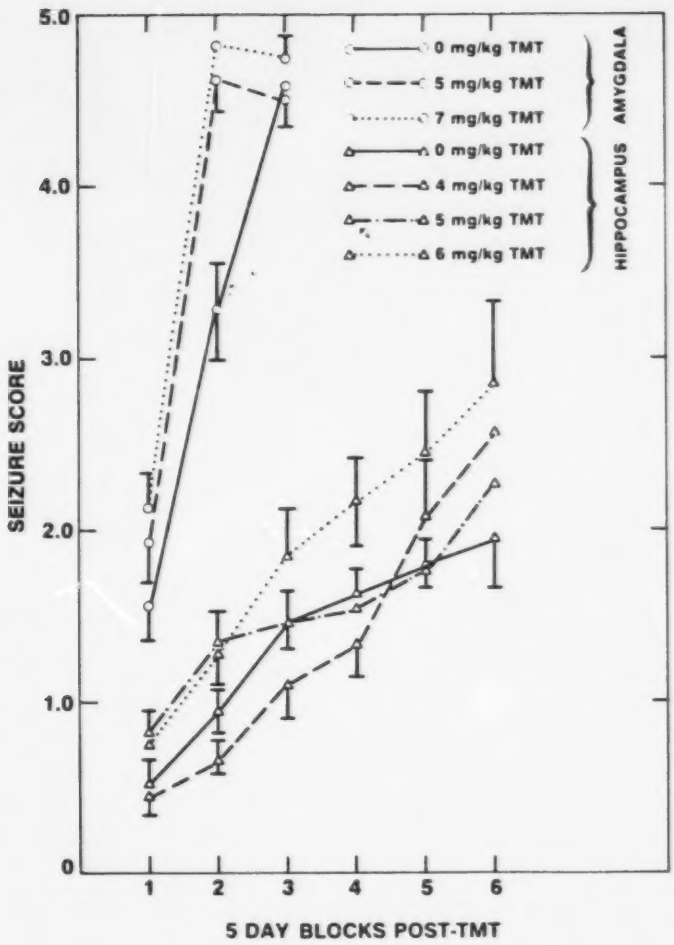

5.0
4.0
3.0
2.0
1.0
0
SEIZURE SCORE
1
2
3
4
5
6
5 DAY BLOCKS POST-TMT
0 mg/kg TMT
5 mg/kg TMT
7 mg/kg TMT
0 mg/kg TMT
4 mg/kg TMT
5 mg/kg TMT
6 mg/kg TMT
HIPPOCAMPUS
AMYGDALA

Fig 19

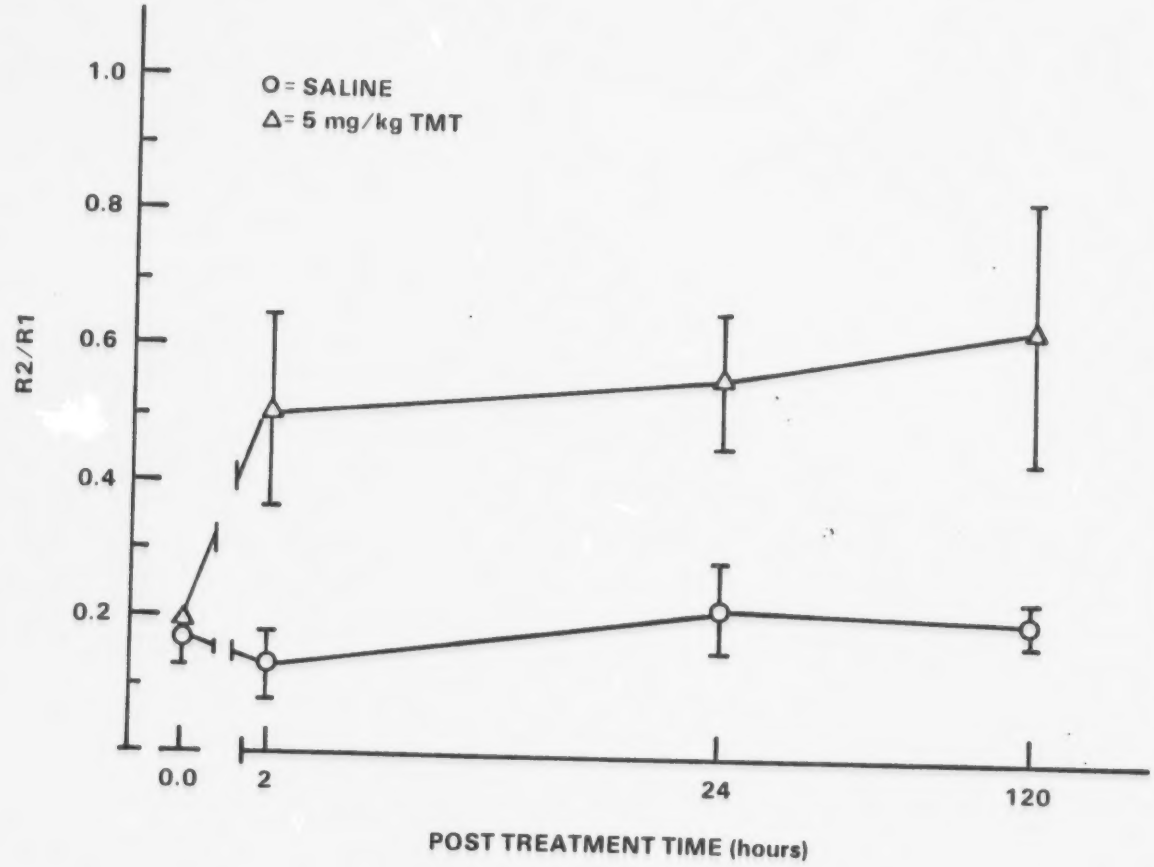

O = SALINE
Δ = 5 mg/kg TMT
R2/R1
1.0
0.8
0.6
0.4
0.2
0.0
2
24
120
POST TREATMENT TIME (hours)

FIG 20

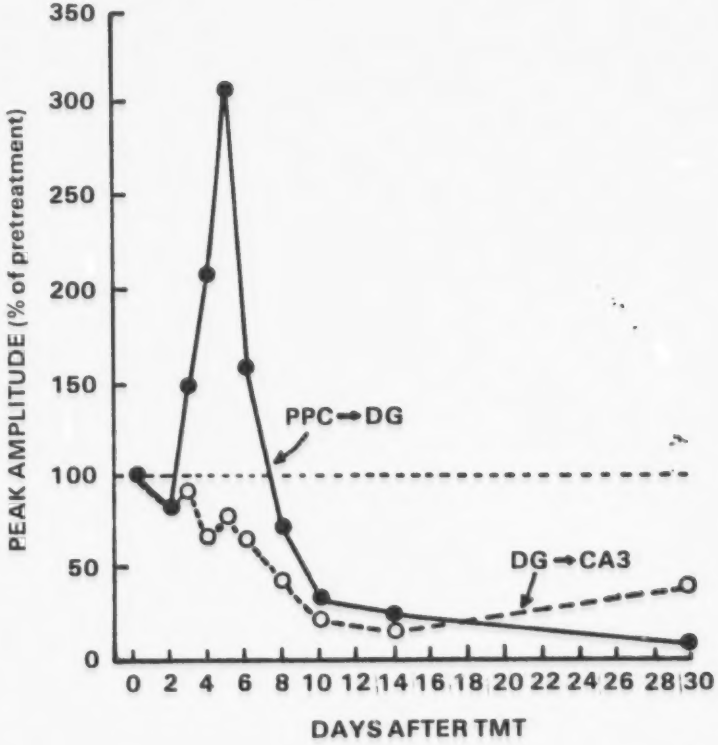

350
300
250
200
150
100
50
0
PEAK AMPLITUDE (% of pretreatment)
PPC→DG
DG→CA3
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
DAYS AFTER TMT

FIG 21